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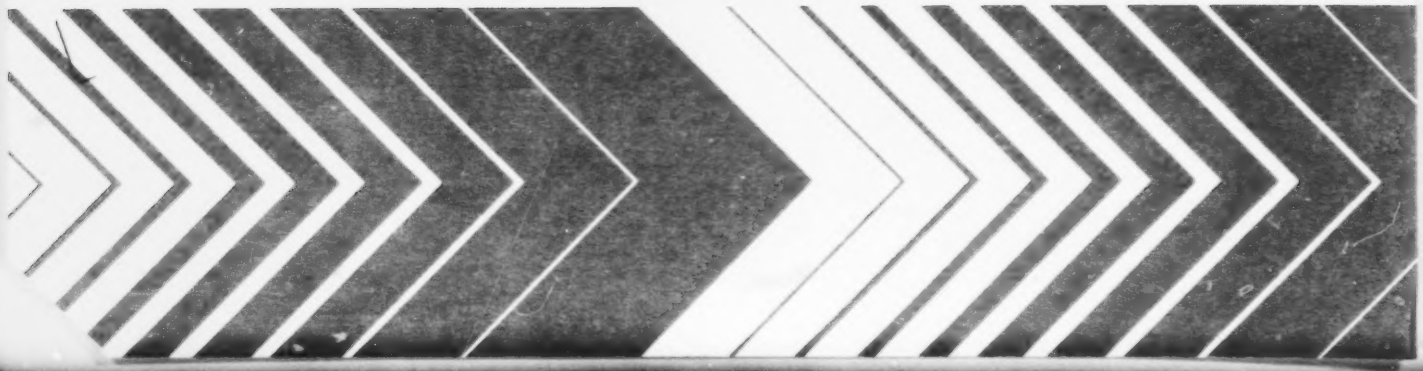

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16. ABSTRACT To optimize the sensitivity of infrared measurements of gas chromatographic effluents, the optimal dimensions for the light-pipe gas cells were first calculated. The transmittance of light-pipes with these optimized dimensions is so high that the signal-to-noise ratio of the single-beam interferograms measured using a mercury cadmium telluride photodetector is limited by digitization noise. To get around this problem, the application of dual-beam Fourier transform infrared spectroscopy was tested and the sensitivity of measurements was four times greater than the single beam measurement. Detection limits of less than 1 ppb were observed when this system was applied to trace organics in water. SCOT columns and shorter light pipes were expected to produce detection limits below 10 ng. The application of dual-beam FT-IR spectroscopy to the on-line identification of peaks eluting from a high performance liquid chromatograph was investigated. Typical detection limits in excess of 10 μg were found, which are too great for general analytical work. Spectra of submicrogram quantities were measured in preliminary work with a system based on diffuse reflectance measurements of deposited solutes on KCl powder. The sensitivity of techniques for the in situ identification of species on specially prepared thin-layer chromatographic plates was improved by the application of programmed multiple development. Detection limits of 100 ng could be achieved in less than 5 seconds data acquisition time, and limits of 10 ng could be achieved after extended signal-averaging.

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AUTOMATED MEASUREMENTS OF INFRARED SPECTRA OF CHROMATOGRAPHICALLY SEPARATED FRACTIONS

by

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Grant No. R804333-01

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FOREWORD

Nearly every phase of environmental protection depends on a capability to identify and measure specific pollutants in the environment. As part of this Laboratory's research on the occurrence, movement, transformation, impact and control of environmental contaminants, the Analytical Chemistry Branch develops and assesses new techniques for identifying and measuring chemical constituents of water and soil.

Gas and liquid chromatographic techniques are effective tools for separating sample components to identify and measure trace organic pollutants in water. The mass spectrometer, coupled to a gas chromatograh, has proved highly successful as a detector for identifying and measuring the separated components that are volatile enough to pass through a gas chromatograph. Significant problems, however, are sometimes associated with identifying certain compounds using mass spectrometry. This report examines the development of an alternative method, Fourier transform infrared spectroscopy, that shows promise for resolving some of these problems and for improving our ability to identify less volatile water pollutants (using high pressure liquid chromatography) at the milligram per liter or microgram per liter level.

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ABSTRACT

This research project was initiated with the overall objective of improving the sensitivity of the interface of a Fourier transform infrared (FT-IR) spectrometer with both gas and high performance liquid chromatographs for the on-line measurement of the spectra of eluting species. These devices have been applied to the identification of trace organic water pollutants.

To optimize the sensitivity of infrared measurements of gas chromatographic effluents, the optimal dimensions for the light-pipe gas cells were first calculated. The transmittance of light-pipes with these optimized dimensions is so high that the signal-to-noise ratio of single-beam interferograms measured using a mercury cadmium telluride photodetector is limited by digitization noise. To get around the problem, the application of a dual-beam FT-IR spectroscopy was tested and the sensitivity of measurements was four times greater than the corresponding single-beam measurement. It is probable that the sensitivity of dual-beam measurements is limited by the poor reproducibility of the interferograms and the nonlinearity of the detector response.

This system was applied to the identification of trace organics in water after extraction using neutral polystyrene resins, elution by diethyl ether and separation by gas-liquid chromatography using 1/8-in. o.d. packed columns. Detection limits of about 250 ng were achieved.

The system was then redesigned for increased sensitivity by reducing the volume of peaks eluting from the chromatograph through the use of support-coated open-tubular columns and shorter light-pipes. The system was not quite completed by the end of the project period, but detection limits below 10 ng could be forecast.

The application of dual-beam FT-IR spectroscopy to the on-line identification of peaks eluting from a high performance liquid chromatograph was investigated. Typical detection limits in excess of 10 μg were found, which are too great for general analytical work. The feasibility of semi-continuous measurements of liquid chromatographic effluents after eliminating the solvent was studied. The lowest detection limits were found with a system based on diffuse reflectance measurements of deposited solutes on KCl powder. Spectra of submicrogram quantities were measured in preliminary work.

The sensitivity of techniques for the *in situ* identification of species on specially prepared thin-layer chromatographic plates was improved by the application of programmed multiple development. Detection limits of 100 ng

could be achieved in less than 5 seconds data acquisition time, and limits of 10 ng could be achieved after extended signal-averaging. The application of diffuse reflectance spectroscopy to the identification of species on aluminum-foil backed thin-layer plates was studied, and although submicrogram detection limits were again found, compensation for strong absorption bands of the adsorbent was found to be extremely difficult.

This report was submitted in fulfillment of Grant No. R804333-01 by Ohio University under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period April 1, 1976 to October 31, 1978, and work was completed as of November 30, 1978.

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LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

a.c.	alternating current
ADC	analog-to-digital converter
cfm	cubic feet per minute
d.c.	direct current
DRIFT	diffuse reflectance infrared Fourier transform
FID	flame ionization detector
FT-IR	Fourier transform infrared
GC	gas chromatography
GC-IR	interface between a gas chromatograph and an infrared spectrometer
GC-FT-IR	GC-IR performed using an FT-IR spectrometer without trapping the sample
GC-MS	interface between a gas chromatograph and a mass spectrometer
HPLC	high performance liquid chromatography
i.d.	internal diameter
LC-IR	interface between a liquid chromatograph and an infrared spectrometer
LC-FT-IR	LC-IR performed with an FT-IR spectrometer
MCT	mercury cadmium telluride
o.d.	outside diameter
PMD	programmed multiple development
ppb	parts per billion (μg/l)
ppm	parts per million (mg/l)
SCOT	support-coated open tubular
S/N	signal-to-noise ratio
TCD	thermal conductivity detector
TGS	triglycine sulfate
TLC	thin-layer chromatography
TLC-IR	*in situ* infrared spectrometry of species on TLC plates
WCOT	wall-coated open tubular
μ-ATR	micro attenuated total reflectance

SYMBOLS

α	half-angle of beam
C_{cell}	maximum value of the concentration of sample in the cell
C_{max}	maximum concentration of sample eluting from the chromatograph
D*	specific detectivity

d_f	beam diameter at a focus
d_p	internal diameter of light-pipe
δ	optical retardation in centimeters
$I'(\delta)$	a.c. and d.c. components of the interferogram
$I(\delta)$	a.c. component of interferogram
$I(\nu)$	source intensity as a function of wavenumber
L	length of light-pipe or pathlength of cell
Q_{cell}	maximum quantity of sample in a cell
Q_{total}	total quantity of sample in a chromatographic peak
ν	wavenumber, cm^{-1}
R	reflectance of light-pipe coating
R_f	relative retention distance in TLC
R_ν	reflectance of beamsplitter at wavenumber, ν
R_s	chromatographic resolution
R_∞	diffuse reflectance at infinite depth
t_a	advance unit time in PMD
t_n	development time for the n th cycle in PMD
T_ν	transmittance of beamsplitter at wavenumber, ν
$T(\nu)$	transmittance of sample at wavenumber, ν
T_p	transmittance of light-pipe
θ_ν	phase lag in interferogram for wavenumber, ν
$V_{\frac{1}{2}}$	volume between the half-width points of a chromatographic peak
V_{cell}	volume of a cell

SECTION 1

INTRODUCTION

The rapid identification of trace organic pollutants in water presents one of the more severe problems for environmental analytical chemists today. The usual procedure is to extract the organics from water by some means, and to analyze the extract (often after further concentration) by one or more chromatographic techniques. The most commonly used technique is gas chromatography, although high performance liquid chromatography is being used to an increasingly large extent for less volatile components. Thin-layer chromatography is now used less frequently to identify individual pollutants, but rather to determine the best conditions for their separation by high performance liquid chromatography. Identification of components separated by gas and liquid chromatography can be attempted by comparison of retention times of the unknown peaks with the retention times of standards, but results found in this way are equivocal at best. Spectroscopic identification of chromatographically separated fractions, preferably without trapping each sample, yields more certain identification of each peak.

The on-line identification of gas chromatographic peaks by mass spectrometry (GC-MS) is now a standard method for investigating organic water pollutants, and detection limits in the low nanogram or high picogram range may be obtained with state-of-the-art equipment. The primary disadvantages of GC-MS are (a) that spectra of closely related compounds, especially isomers, are not readily distinguished, (b) spectra may differ from spectra in reference compilations because of different ionization conditions in the spectrometer, and (c) reference spectra of the unknown sample may not be available, in which case the spectrum must be interpreted *a priori*. An alternative, or supplementary, general method for the on-line identification of gas chromatographic peaks would be most beneficial for studying water pollutants. Fourier transform infrared (FT-IR) spectroscopy may be the most promising alternative, but until recently gas chromatographic peaks have not been identified at submicrogram levels by FT-IR spectroscopy. One objective of this study was to develop methods for increasing the sensitivity of on-line FT-IR methods for identifying organic water pollutants separated by gas chromatography (GC-FT-IR).

Components of mixtures separated by high performance liquid chromatography are less easily identified by mass spectrometry because the samples are generally less volatile than samples separated by gas chromatography and because they are more difficult to separate from the mobile phase. FT-IR spectroscopy therefore may be presented as a viable alternative for the on-line identification of species separated by this technique, and a second objective of this project was the development of FT-IR methods for identifying

species separated by liquid chromatography with practical detection limits of less than one microgram.

The final objective was the refinement of FT-IR methods for identifying species separated by thin-layer chromatography, without removing the sample from the plate. No general alternative method to infrared spectroscopy is currently in use, although ultraviolet-visible spectrometry and fluorescence spectrometry may both be used for certain types of compounds.

Each of these FT-IR methods could be of real importance in identifying water pollutants at the parts-per-million or parts-per-billion level.

SECTION 2

CONCLUSIONS

Calculations have shown that the optimum dimensions for "light-pipe" gas-cells, through which the effluent from a gas chromatograph is passed for the FT-IR measurement, would cause the transmittance of the cell to be so great that if interferograms were measured using a conventional rapid-scanning interferometer and a mercury cadmium telluride photodetector, the signal-to-noise ratio near the point of stationary phase would be so great that the limiting source of noise would be the digitizing system rather than the detector. To avoid this problem, dual-beam FT-IR spectroscopy can be used to eliminate the large signal due to the source and leave only the small signal caused by the absorbing sample. Even though the stability of our interferometer was not good, and the response of the detector was nonlinear at the high light levels of this experiment, the detection limits of dual-beam FT-IR spectroscopy for the on-line identification of gas chromatographic peaks were about four times lower than were found in the corresponding single-beam measurement. When the system was applied to the identification of trace organics in water after extraction by neutral polystyrene resins, detection limits of less than one part-per-billion were observed. The system was redesigned to be compatible with high resolution gas chromatography using capillary columns, and preliminary results indicate that detection limits of less than 10 nanograms of strongly absorbing samples will ultimately be achieved.

The on-line identification of submicrogram amounts of samples separated by high performance liquid chromatography without separating the solvent proved to be impossible even using dual-beam FT-IR spectroscopy; detection limits of about 10 μg for strong absorbers and 100 μg for weak absorbers were found. Several semi-continuous methods of solvent removal followed by the rapid measurement of the FT-IR spectra of each solute were studied. The most successful method to date involves a preliminary concentration step, in which the effluent from the chromatograph is sprayed into a heated tube, followed by a deposition step, where the concentrated solution is sprayed onto a small cup containing powdered potassium chloride. After the peak is eluted the cup is automatically transferred to the focus of a diffuse reflectance attachment of an FT-IR spectrometer. Submicrogram detection limits for nonvolatile compounds are found with the technique.

Transmittance spectra of the sample spots on developed thin-layer chromatography (TLC) plates can be measured provided that the adsorbent is deposited on a silver chloride backing. The effect of scattering by the adsorbent can be minimized by treating the plate with an oil whose refractive index matches that of the adsorbent. Submicrogram detection limits can be

achieved in very short measurement times if the spot size is minimized by the use of programmed multiple development TLC, thereby demonstrating the feasibility of an automated FT-IR scanner for TLC plates. The use of diffuse reflectance FT-IR also appears to be a promising method for the identification of sample spots on glass or aluminum-foil backed TLC plates; again submicrogram detection limits have been observed.

SECTION 3

RECOMMENDATIONS

The most important work to be finished in this study is the development of the device for the semi-continuous identification of species eluting from a high performance liquid chromatograph based on diffuse reflectance infrared Fourier transform spectroscopy. Several parts of this device require optimization. The propellant gas flow rate, temperature and dimensions of the preconcentrator, the method of applying the concentrated solution to the alkali halide powder, and the particle size, depth and temperature of the powder all require further investigation to optimize the performance of this device.

The true potential of dual-beam FT-IR spectroscopy should be studied after stabilizing the interferometer with the fast retrace electronics. For the study of gas chromatographic effluents, the increased stability should lead to improvements in phase correction, reduction in the effect of atmospheric interferences, and probably a small increase in sensitivity. For the study of liquid chromatographic effluents, the increased interferometer stability should lead to substantially improved solvent compensation and a proportionately greater increase in sensitivity than for the gas chromatography work.

Further studies on improving the performance of the system designed to measure the spectra of compounds eluting from gas chromatographs using capillary columns are required. A dual-beam FT-IR system with 2mm diameter light-pipes should be built, so that its performance can be tested against the corresponding measurements made using 3mm diameter light-pipes. After optimizing the performance of a "breadboard" system, an all-glass system should be constructed to allow all organic compounds to be measured without decomposition, preferably using a nondestructive photoionization detector.

Ideally, a combined system for simultaneously identifying species eluting from a gas chromatograph and a high performance liquid chromatograph using two interferometers controlled by a single data system should be built. This system should be applied to the identification of the organic pollutants in actual water samples taken from a variety of sources.

Finally our preliminary investigation into the application of diffuse reflectance infrared spectroscopy for the elucidation of a wide variety of environmental problems should be expanded. Problems to be studied should include not only the identification of species on TLC plates, but also the investigation of organic compounds, especially pesticides, sorbed onto clays and other minerals, and even the potential for qualitative and quantitative analysis of organics sorbed onto charcoal and other adsorbents from industrial atmospheres.

SECTION 4

THEORETICAL CONSIDERATIONS IN GC-FT-IR

OPTIMAL LIGHT-PIPE VOLUME

To optimize GC-FT-IR sensitivity, it is necessary to consider the performance of the chromatograph, the spectrometer and the light-pipe gas cell through which the effluent from the chromatograph is being passed while its spectrum is being measured. The dimensions of the light-pipe are of critical importance, and as the first part of this project, the optimum dimensions for GC-IR light-pipes were calculated; these results have been published (1) and are summarized below. Other factors which have been studied include the data collection trigger, the performance of the spectrometer and detector, and the chromatographic separation. The conclusions from these calculations are summarized in the following subsections of this section of the report.

It is intuitively apparent that, if the volume of the light-pipe, V_{cell}, is much larger than the volume between the half-width points of any chromatographic peak, $V_{\frac{1}{2}}$, two peaks which are just resolved by the chromatograph could be present simultaneously in the light-pipe. In this case the resolution of the chromatograph could be seriously degraded. In addition the maximum value of the average concentration of the sample in the cell, C_{cell}, will be substantially less than the maximum concentration of the sample in the peak, C_{max}, as it elutes from the column. On the other hand, if $V_{cell} \ll V_{\frac{1}{2}}$, the maximum quantity of sample in the cell, Q_{cell}, will be much smaller than the total quantity of sample in the peak, Q_{total}, even though C_{cell} approximates C_{max}.

The variation of C_{cell}/C_{max} and Q_{cell}/Q_{total} with $V_{cell}/V_{\frac{1}{2}}$ was calculated for triangular GC peaks, and the result is shown in Figure 1. Calculations which assume a Gaussian GC peak shape yield a very similar plot. The intersection of these two curves occurs at the optimum value of $V_{cell}/V_{\frac{1}{2}}$, and it can be seen from Figure 1 that this occurs when $V_{cell}=V_{\frac{1}{2}}$. Under these conconditions the GC-IR peak (i.e. the variation of the absorbance of a sample in the light-pipe as a function of time) should be wider than the GC peak by approximately 50%. We believe that this small degradation in resolution is a small price to pay for optimizing the sensitivity of on-the-fly GC-FT-IR measurements.

To quantitate the effect of this peak broadening on spectral interferences if $V_{cell} = V_{\frac{1}{2}}$, the cross-contamination of neighboring GC peaks of equal concentration and half-width, separated with a chromatographic resolution, R_s, was calculated. R_s is defined as the ratio of the difference in the retention volumes of the peaks to the average of the half-widths (2). Figures 2 and 3 show the extent of interference by neighboring triangular peaks for $R_s = 1.0$ and 0.8, respectively. It can be seen from these figures that, if the data are only collected between the half-width points of the peaks (after allowing a suitable delay), the amount of cross-contamination is negligibly small, unless the interfering peak is a much stronger infrared absorber than the peak of interest. Similar results may again be shown for Gaussian GC peaks, the extent of cross-contamination being about 50% greater than for triangular peaks.

OPTIMAL DATA COLLECTION TRIGGER

Several methods of data collection for GC-FT-IR have been proposed. In the simplest each interferogram is stored separately; however, such a scheme

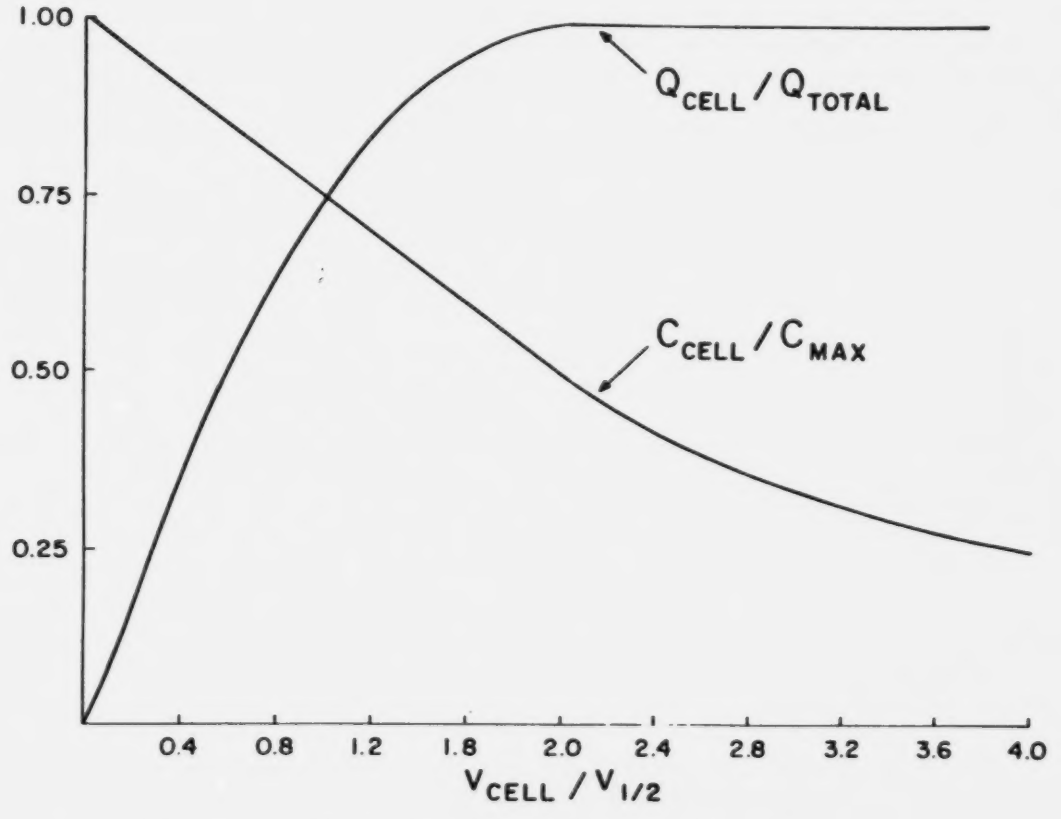

Figure 1. Variation of Q_{cell}/Q_{max} and Q_{cell}/Q_{total} with $V_{cell}/V_{\frac{1}{2}}$ for triangular GC peaks.

implies that 3600 interferograms of 1 sec. scan time must be stored for a chromatogram of 1 hour duration. This in turn imples a very large data system. For 4 cm^{-1} resolution interferograms, it is usual to collect 4K data points in double-precision, so that about 2.6×10^6 words of storage are needed. In addition the data would take an inordinately long time to compute, even with a faster transform than is available on current commercial FT-IR spectrometers. It is possible to reduce the amount of data storage by co-adding several successive interferograms, but this approach can degrade the chromatographic resolution and still does not completely solve the problem of an excessive data storage requirement.

A superior approach is to collect data only when a sample is present in the light-pipe at a concentration which will yield spectra at a sufficiently high signal-to-noise ratio (S/N) to permit several absorption bands to be identifiable. The first GC-FT-IR systems used a sensor which permitted interferograms to be signal-averaged during the period when the signal from the GC detector exceeded a certain threshold, after allowing for a delay time to take into account the time difference between the maximum signal at the GC detector and the maximum sample concentration in the light-pipe. For successful operation, this system relied on the operator to set the threshold at a value which was large enough to allow small peaks (which would not yield an identifiable spectrum) to pass through the light-pipe without being measured, but small enough to permit a reasonably large number of interferograms to be averaged across any given GC peak. A further disadvantage of this type of trigger is found for partially resolved GC peaks for which the

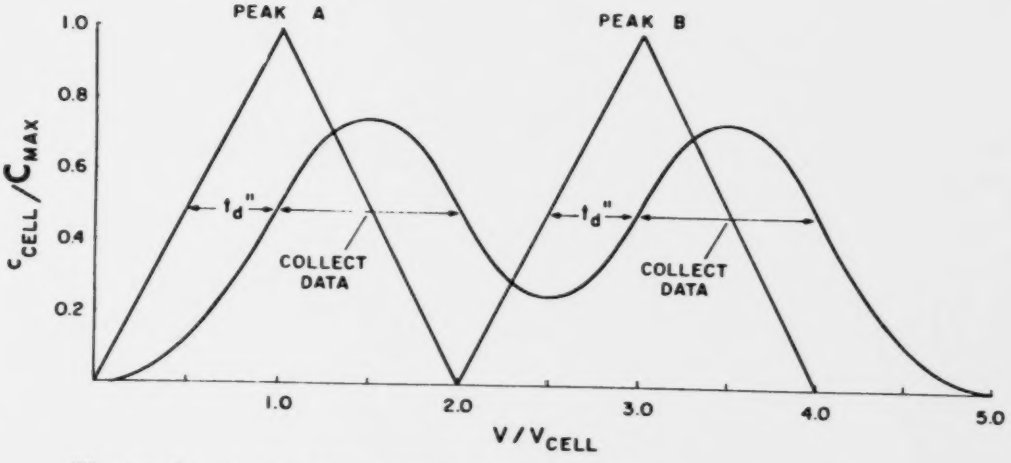

Figure 2. Peak spreading in a cell with $V_{cell} = V_{\frac{1}{2}}$ for triangular GC peaks separated by 2 $V_{\frac{1}{2}}$ ($R_s = 1.0$). The triangular peaks represent the signal at the GC detector, and the smooth curve gives the sum of the values of Q_{cell} for A and B. Data should be collected between the half width points of the GC peak after a time delay, t_d''.

dip between the peaks always exceeds the threshold; in this case, signal-averaging continues across the unresolved peaks. In addition, a drift of the baseline of the chromatogram can cause data acquisition to be initiated and continued until the operator resets the baseline below the threshold.

It can be shown (1) that a close approach to the optimum sampling trigger for GC-FT-IR could be actuated by the inflexion points of the Gaussian GC peaks. It is relatively simple to electronically sense these points of inflexion using the zero-crossings of the second derivative of the GC signal. We calculated that if signal-averaging were performed for this period of time, the S/N would be greater than 90% of the maximum possible value. If the GC peaks are separated to the extent that there is even the smallest dip between the peaks, interferograms for each such resolved peak will be averaged separately. One of the disadvantages of a "second derivative trigger" is that it is possible to initiate and terminate data collection by noise, and one method of discriminating against the effect of noise is to actuate the second derivative trigger only when the signal exceeds a certain threshold value. A trigger of this type, however, would still be adversely affected by baseline drift.

An even better system would be a microprocessor-based trigger which would initiate data collection using a second-derivative trigger after checking for a period of time double or triple the time constant of the GC detector amplifier to check if a second zero crossing (presumably generated

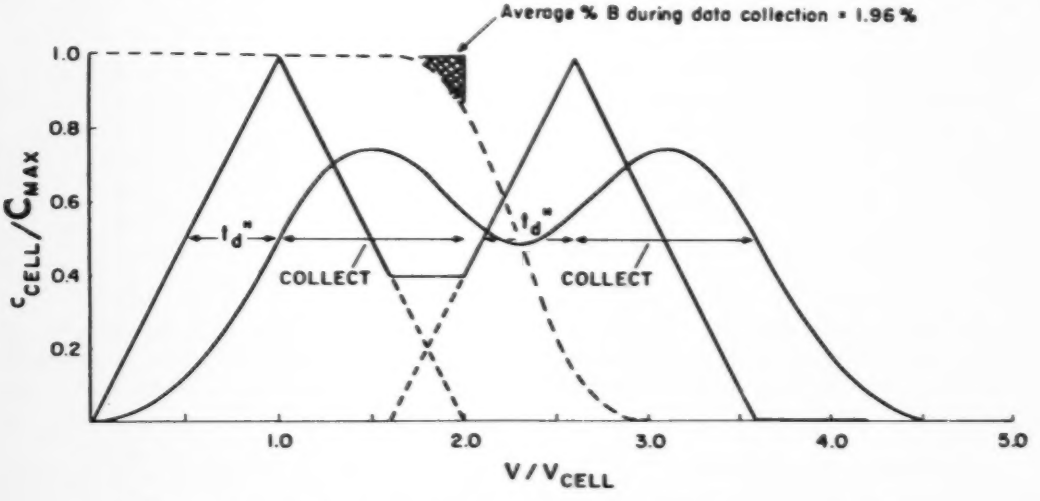

Figure 3. Peak spreading in a cell with $V_{cell} = V_{\frac{1}{2}}$ for triangular GC peaks separated by 1.6 $V_{\frac{1}{2}}$ ($R_s = 0.8$). The upper broken line represents the ratio of the quantity of peak A to the total sample quantity in the light-pipe. If data acquisition occurs between the half-width points of each peak, after the optimum time delay, t_d'', the average percentage contamination of peak A by peak B (as shown by cross-hatching) is less than 2%.

by noise) has occurred. We are currently building such a system based on the KIM-1 microcomputer (MOS Technology, Norristown, PA). Because of the difficulties involved with 60 Hz pick-up on the microvolt level signals in this device, we have not yet been able to construct a working trigger.

OPTIMAL LIGHT-PIPE DIMENSIONS

If an extremely low noise infrared spectrophotometer with a very wide dynamic range was available for GC-IR measurements, it is apparent that the light-pipe should be as long and narrow as possible, consistent with its volume, V_{cell}, being equal to $V\frac{1}{2}$ as discussed above. However the baseline noise level and dynamic range of rapid-scanning Fourier transform spectrometers are by no means ideal, and it is necessary to consider the trade-off between the length, L, and diameter, d_p, of light-pipes on GC-IR sensitivity. Mantz (3) has studied the problem from theoretical grounds and has derived an equation relating the transmittance of a light-pipe, T_p, to the reflectivity of the coating, R, the half-angle of the incoming beam, α, and the length and diameter of the light-pipe:

$$T_p = R^{(-2L/d_p)} \ln \cos \alpha$$

If the reflectivity of the coating is known, the optimum dimensions of the light-pipe could be calculated in this way, but coating imperfections and the variation of R with polarization and incidence angle make this approach less than useful in practice.

We developed a more practical basis for determining T_p. By using a single-beam GC-FT-IR arrangement in which the diameter of the focused beam at the entrance of the light-pipe, d_f, was 0.3 cm, we were able to derive an empirical expression for the transmittance of any light-pipe used on this system with $d_p < 0.3$ cm:

$$T_p = 0.9 \left(\frac{d_p}{d_f}\right)^2 \quad 0.32^{(0.006L/d_p)}$$

In this expression the first term (0.9) is due to the reflection loss at the windows, the second term is due to vignetting of the beam at the entrance to the light-pipe, and the third term is due to reflection losses down the light-pipe. If $d_p > d_f$, the second term is omitted.

The noise level of the spectral baseline (100% line) should be inversely proportional to T_p, and for weakly absorbing bands (typical of the GC-FT-IR experiment) the sample absorptance should be approximately proportional to the absorbance which is proportional to L. Thus the S/N should be proportional to the product of L and T_p. Plots of LT_p vs L for different values of V_{cell} revealed that the maximum usually occurred with $d_p = d_f$, and that the transmittance of the cell at maximum efficiency is usually greater than 20%, see Figure 4. This rather high transmittance has important implications for FT-IR measurements in which a mercury cadmium telluride (MCT) detector is used instead of the conventional triglycine sulfate (TGS) detector, as discussed in the next section.

SPECTROMETER PERFORMANCE

Interferometer

Commercial FT-IR spectrometers usually have a 50 mm aperture and a Ge:KBr beamsplitter. In theory there is little one should be able to do to improve the performance with the exception of increasing the aperture (to increase the optical throughput) or changing the scan speed of the moving mirror. The former operation cannot be performed on any interferometer once it is built, while the scan speed cannot be changed on the majority of commercially available interferometers. However it should be recognized that the nature of the detector determines the optimum scan speed. The TGS detector works best at relatively low modulation frequencies, thereby necessitating a slower scan speed than if the MCT detector is being used.

In a comparative study of the relative performance of the spectrometer being used for this project at Ohio University, a Digilab Model FTS-14 (Digilab, Inc., Cambridge, MA) which was constructed in 1973, and a recent instrument with the same specifications, it was found that the modern

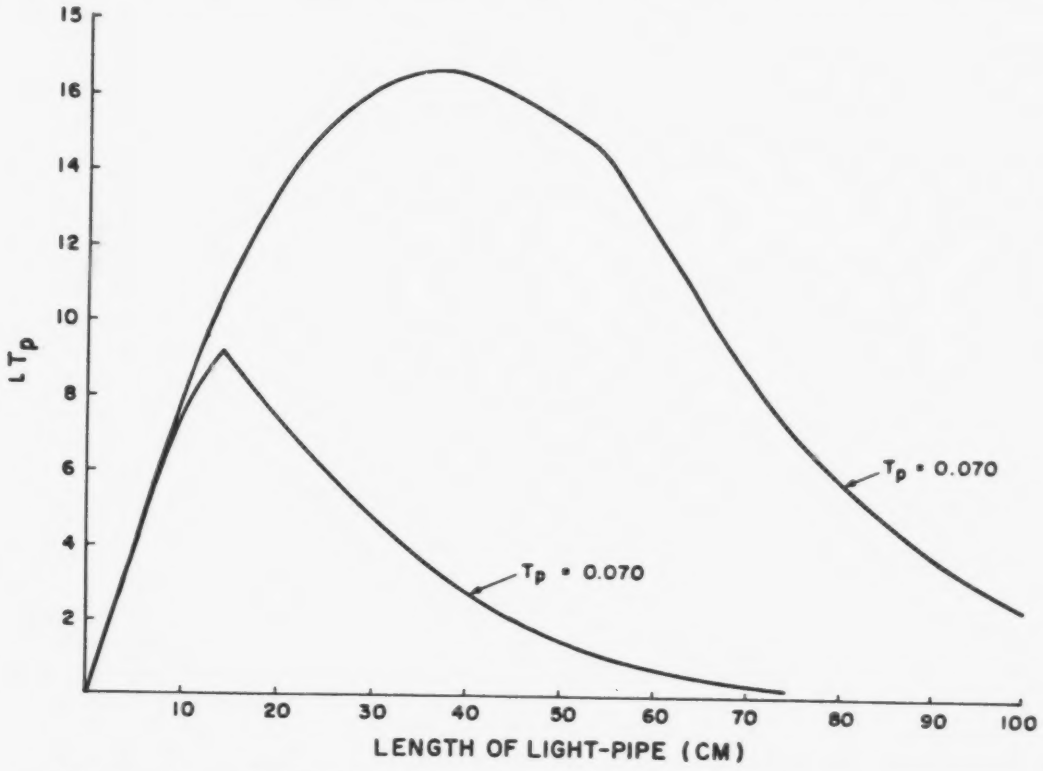

Figure 4. Calculated variation of LT_p as a function of light-pipe length for 4-ml (upper trace) and 1-ml (lower trace) light-pipes. When T_p exceeds 0.07, the S/N of measurements made with an MCT detector is limited by digitization noise rather than detector noise.

instrument gave substantially superior performance (4), especially at high frequency. It is believed that the improvements to commercially available spectrometers are due to improvements in beamsplitter design and in the signal electronics. Comparison between the work described in this report and published work performed on later instruments should be made with the decreased performance of the early instruments in mind.

Source

The two commercially available sources for mid infrared FT-IR spectrometers are the nichrome wire and the globar. Nichrome wire sources operate at a lower temperature than globars and, unlike globars, do not require water-cooling; however the emitted energy from a nichrome wire source is somewhat less than that from a globar at all wavenumbers.

A compromise between the convenience and cheapness of nichrome wire sources and the performance of a globar is found in the Nernst glower. The Nernst glower operates at a higher temperature than the globar but does not require water cooling. The primary disadvantage of the Nernst glower is its low emissivity above about 2500 cm^{-1}, but it is an excellent source for the region from 2000 to 100 cm^{-1}. Comparative spectra of the Nernst glower and the nichrome wire source measured identically in all other respects are shown in Figure 5. The nichrome wire was an original component of our Digilab FTS-14 spectrometer and the Nernst glower was an x37 type (Perkin-Elmer Corp., Norwalk, CT) powered with the original d.c. supply of the FTS-14.

Detector

The TGS pyroelectric bolometer is usually used on mid infrared FT-IR spectrometers. The TGS detector is not as sensitive as conventional thermal detectors such as the thermocouple, but it has the advantage of a very short response time which is necessary because the scan speed of the moving mirror of a rapid-scanning interferometer has to be kept high enough to keep the S/N of the interferogram near the point of stationary phase below the dynamic range of the analog-to-digital converter (ADC) being used to digitize the signal. For an interferometer with a 50 mm aperture set up for mid infrared spectrometry (4000-400 cm^{-1}) using a TGS detector, the scan speed to keep the S/N below the dynamic range of a 15-bit ADC is approximately 3 mm/sec.

The average detectivity, D*, of the MCT detector is about twenty times greater than that of the TGS detector and, if an MCT detector were directly substituted for the TGS without attenuating the source in any way, the scan speed of the interferometer mirror would have to be increased by a factor of $(20)^2$ to avoid digitization noise. Even though the MCT detector could still respond to all frequencies in the interferogram, the sampling frequency would have to be raised to about 1 MHz and the data acquisition rate would far exceed the capability of a state-of-the-art 15-bit ADC. Even if a light-pipe with $T_p = 0.25$ were interposed between the interferometer and the light-pipe, a sampling frequency in excess of 100 kHz is required which still exceeds the maximum rate for a 15-bit ADC.

To optimize the sensitivity of GC-FT-IR measurements it is still necessary to use the most sensitive detector possible, but under conditions where the signal is limited by detector noise rather than digitization noise and without changing the scan speed of the interferometer (so that the limits of the band pass filter in the amplifier do not have to be changed from the values currently available on commercial FT-IR spectrometers). To achieve this goal it is necessary to eliminate the very high signal near the point of stationary phase of the interferogram, which is due to the intense continuous spectrum of the source, while retaining the small modulations due to the sample. Some possible ways of achieving this end are as follows:

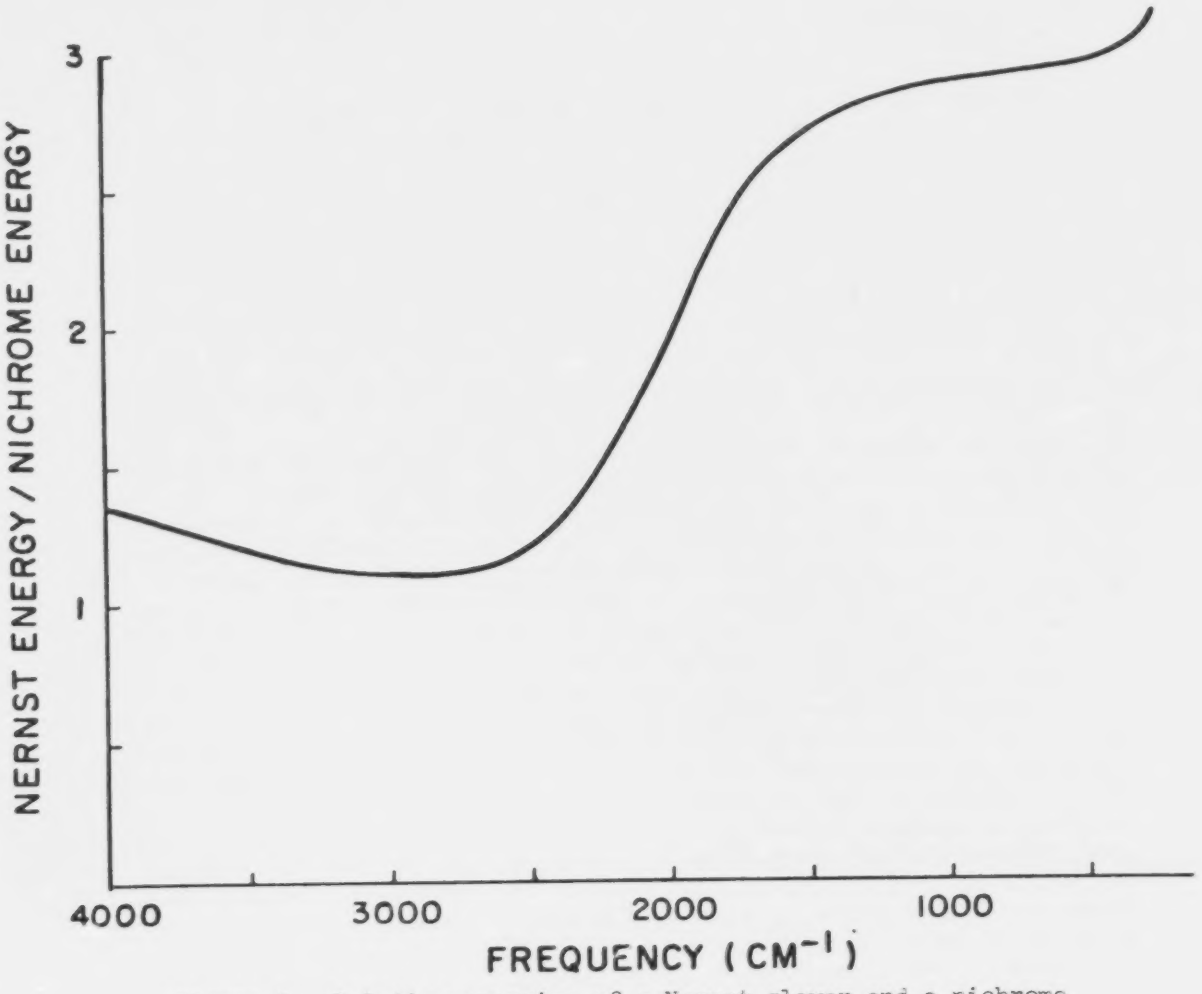

Figure 5. Relative energies of a Nernst glower and a nichrome wire infrared source as a function of wavenumber. The effect of the low emissivity of the Nernst glower around 3000 cm^{-1} is apparent.

a. Reducing the spectral range of the measurement, either by an optical filter or by reducing the temperature of the source. Either of these two courses of action would reduce the multiplex advantage of FT-IR spectrometry and is less than desirable.

b. By "clipping" or "blanking" the interferogram (5). This technique severely degrades the photometric accuracy of the measurement and creates a very poor baseline on which it is extremely difficult to observe weak absorption bands.

c. By "chirping" the interferometer (6,7). This technique increases transform time substantially and has never been tested for interferograms of high dynamic range since it is believed that the S/N of spectra computed from chirped interferograms is less than that of spectra computed from unchirped or only slightly chirped interferograms.

d. Through the use of dual-beam, or optical subtraction, techniques (8-11). The apparent advantages of dual-beam FT-IR appear to substantially outweigh the potential disadvantages and this was the technique by which we decided to attempt to study the use of optimized light-pipes for high sensitivity GC-FT-IR. The theory of dual-beam FT-IR is described in Section 5, together with descriptions of our optical system and comparative results. The application to GC-FT-IR is described in Section 6.

OPTIMAL CHROMATOGRAPHIC PERFORMANCE

A truly versatile GC-FT-IR system should be able to be used to identify peaks separated on both packed and capillary columns. However one important goal of GC-FT-IR research in general, and this project in particular, is to reduce detection limits (in terms of nanograms of a given component injected onto the column) to the maximum possible extent. It can be shown (1) that the small $V_{\frac{1}{2}}$ of peaks separated on capillary columns allows one to use cells of smaller volume than is necessary for packed columns, and that smaller detection limits should therefore be predicted. It should be noted that the increased capacity of packed columns may sometimes offset the low detection limits obtainable with capillary columns by allowing a greater volume of the analyte to be injected onto the column initially. This effect is particularly important for wall-coated open tubular (WCOT) capillary columns, onto which it is customary to inject about 10 nl of the analyte solution. However the capacity of support-coated open tubular (SCOT) columns is usually sufficiently large that microgram quantities of components can be injected without degrading the performance of the column. The use of SCOT columns therefore presents a good compromise between the resolution of WCOT columns and the capacity of packed columns, and these columns should allow the lowest detection limits of GC-FT-IR to be attained.

In this project we first attempted to optimize GC-FT-IR performance using 1/8-in. o.d. packed columns, since this is the type of column most generally used in gas chromatography today. Towards the end of the project we fitted the chromatograph with a SCOT column to determine the extent to which GC-FT-IR detection limits would be reduced. The results are described in Section 6.

SECTION 5

DUAL-BEAM FOURIER TRANSFORM INFRARED SPECTROMETRY

THEORY

If the collimated input beam of a conventional Michelson interferometer enters at the usual angle of 45° to the plane of the beamsplitter, one of the emerging beams returns to the source and is not measured; this beam is usually called the "reflected beam", and is denoted by the subscript B in this report. The other beam (the "transmitted beam" which is denoted by the subscript A in this report) is passed through the sample and is then measured at the detector. If the beam enters at an angle other than 45° to the beamsplitter both the transmitted and the reflected beams could be detected.

The intensity of the interferogram of the transmitted beam, $I'(\delta)_A$, is given as a function of retardation, δ cm, by the equation:

$$I'(\delta)_A = 2\int_0^{+\infty} R_\nu T_\nu \, I(\nu).d\nu + 2\int_0^{+\infty} R_\nu T_\nu \, I(\nu) \cos(2\pi\nu\delta + \theta\nu).d\nu$$

where $I(\nu)$ is the intensity of the source at wavenumber νcm^{-1},
R_ν is the reflectance of the beamsplitter at νcm^{-1},
T_ν is the transmittance of the beamsplitter of νcm^{-1},
and θ_ν is a small wavenumber dependent phase angle.

The modulation of the reflected beam is 180° out-of-phase with the transmitted beam, and it can be shown that:

$$I'(\delta)_B = \int_0^{+\infty} (R_\nu^2 + T_\nu^2) \, I(\nu).d\nu - 2\int_0^{+\infty} R_\nu T_\nu \, I(\nu) \cos(2\pi\nu\delta + \theta_\nu).d\nu$$

If both beams are somehow measured at the same detector, the signal is given by:

$$I'(\delta)_A + I'(\delta)_B = 2\int_0^{+\infty} R_\nu T_\nu \, I(\nu) \, d\nu + \int_0^{+\infty} (R_\nu^2 + T_\nu^2) \, I(\nu).d\nu$$

$$= \int_0^{\infty} (R_\nu + T_\nu)^2 \, I(\nu).d\nu$$

For a non-absorbing beamsplitter:

$$R_\nu + T_\nu = 1$$

Therefore

$$I'(\delta)_A + I'(\delta)_B = \int_0^{+\infty} I(\nu).d\nu$$

i.e. the total energy entering the interferometer equals the total energy emerging and the sinusoidal components of $I'(\delta)_A$ and $I'(\delta)_B$ are nulled out. In rapid-scanning mid infrared FT-IR spectrometers, the amplifier for the detector is a.c. coupled so that only the sinusoidal (modulated) component of the interferogram is measured. Thus if no sample is in either beam, no a.c. signal would be measured. On the other hand, if a sample of transmittance $T(\nu)$ is placed in the reflected beam:

$$I'(\delta)_B = \int_0^{\infty} (R_\nu^2 + T_\nu^2)T(\nu)I(\nu).d\nu - 2\int_0^{\infty} R_\nu T_\nu T(\nu)I(\nu) \cos(2\pi\nu\delta + \theta_\nu).d\nu$$

If the modulated components of the transmitted and reflected interferograms are denoted by $I(\delta)_A$ and $I(\delta)_B$, respectively, we have that:

$$I(\delta)_A + I(\delta)_B = 2\int_0^{+\infty} R_\nu T_\nu[1-T(\nu)]\, I(\nu) \cos(2\pi\nu\delta + \theta_\nu).d\nu$$

Therefore in this case, the greater the transmittance of the sample, the <u>smaller</u> is the magnitude of the a.c. interferogram. For very small sample quantities, $[1-T(\nu)]$ is usually very small, so that if an MCT detector were directly substituted for a TGS detector without changing the scan speed of the interferometer, the resultant interferogram should not be digitization noise limited.

$T(\nu)$ can be calculated by dividing the measured dual-beam spectrum by the single-beam spectrum, $I(\nu)$, calculated from the transform of $I(\delta)_A$, and subtracting the result from unity.

OPTICAL DESIGN

The optical system (12) that we designed for dual-beam FT-IR is shown in Figure 6. The source was the Nernst glower described in the previous section. The beam from this source was collimated by a 45° off-axis segment of a 75 mm focal-length paraboloidal mirror (Special Optics Corporation, Little Falls, NJ), which provided a beam of the maximum allowed throughput for wavenumbers below 4000 cm^{-1} for measurements made with a resolution, $\Delta\nu > 2$ cm^{-1}. The interferometer was a Model 296 Michelson interferometer, which is a component of the Digilab Model FTS-14 Fourier transform spectrophotometer. The emerging beams were picked off by two front-surface, gold-

coated plane mirrors (Edmund Scientific Corporation, Barrington, N.J.) and focused using two off-axis paraboloidal mirrors identical to the collimating mirror for the source; the foci for the sample and reference beams were separated by 6 inches, which was the separation of the two light-pipes in our preliminary GC-IR set-up, vide infra. The beams were then refocused onto a liquid nitrogen cooled MCT detector of 2mm side (Texas Instruments, Dallas, TX) using two off-axis sections of an ellipsoid of focal lengths 109 and 262 mm (Special Optics Corp.).

The system was designed to allow the detector and the two ellipsoids to be moved back as a unit, so that dual parallel light-pipes could be placed with their entrance apertures at the sample and reference foci, as shown in Figure 7. Without the light-pipes present, this dual-beam FT-IR system could be used for studying conventional solutions at low concentration. If a flow-through cell was placed in the reflected beam and a reference cell of equal pathlength were placed in the transmitted beam (or vice versa), this system could be used for on-line measurements of samples eluting from a high performance liquid chromatograph.

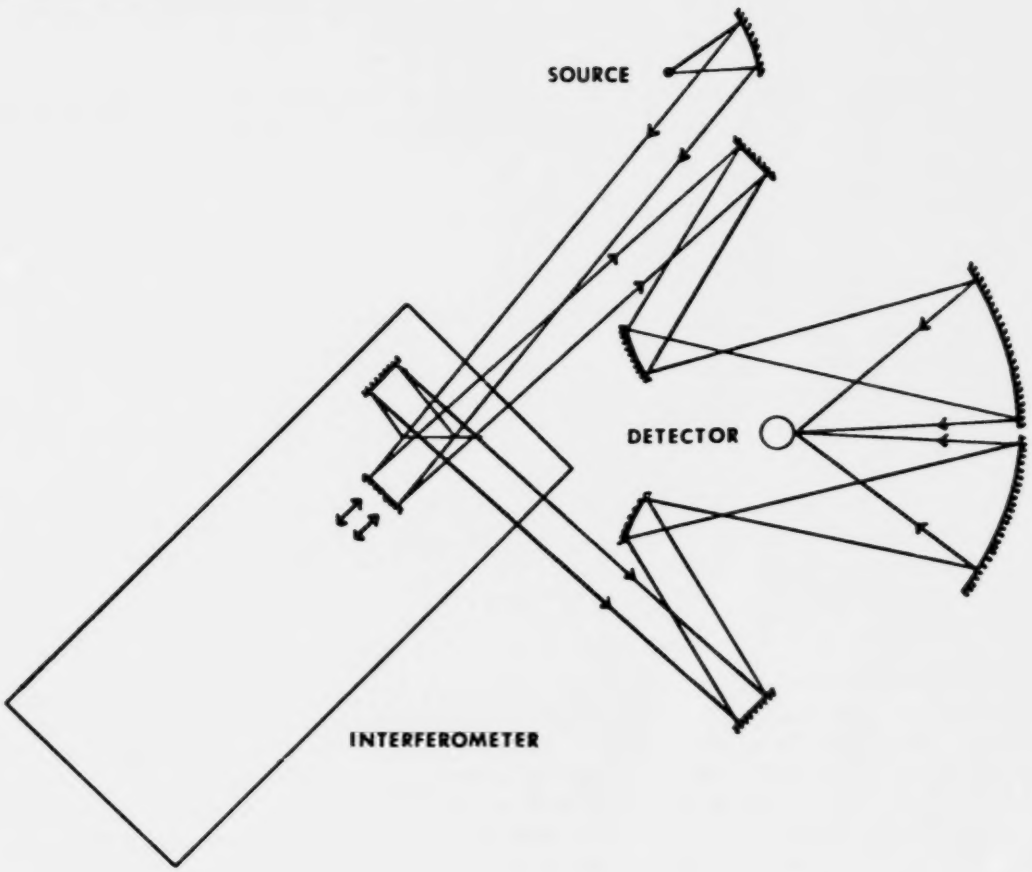

Figure 6. Optical layout for dual-beam FT-IR spectrometry.

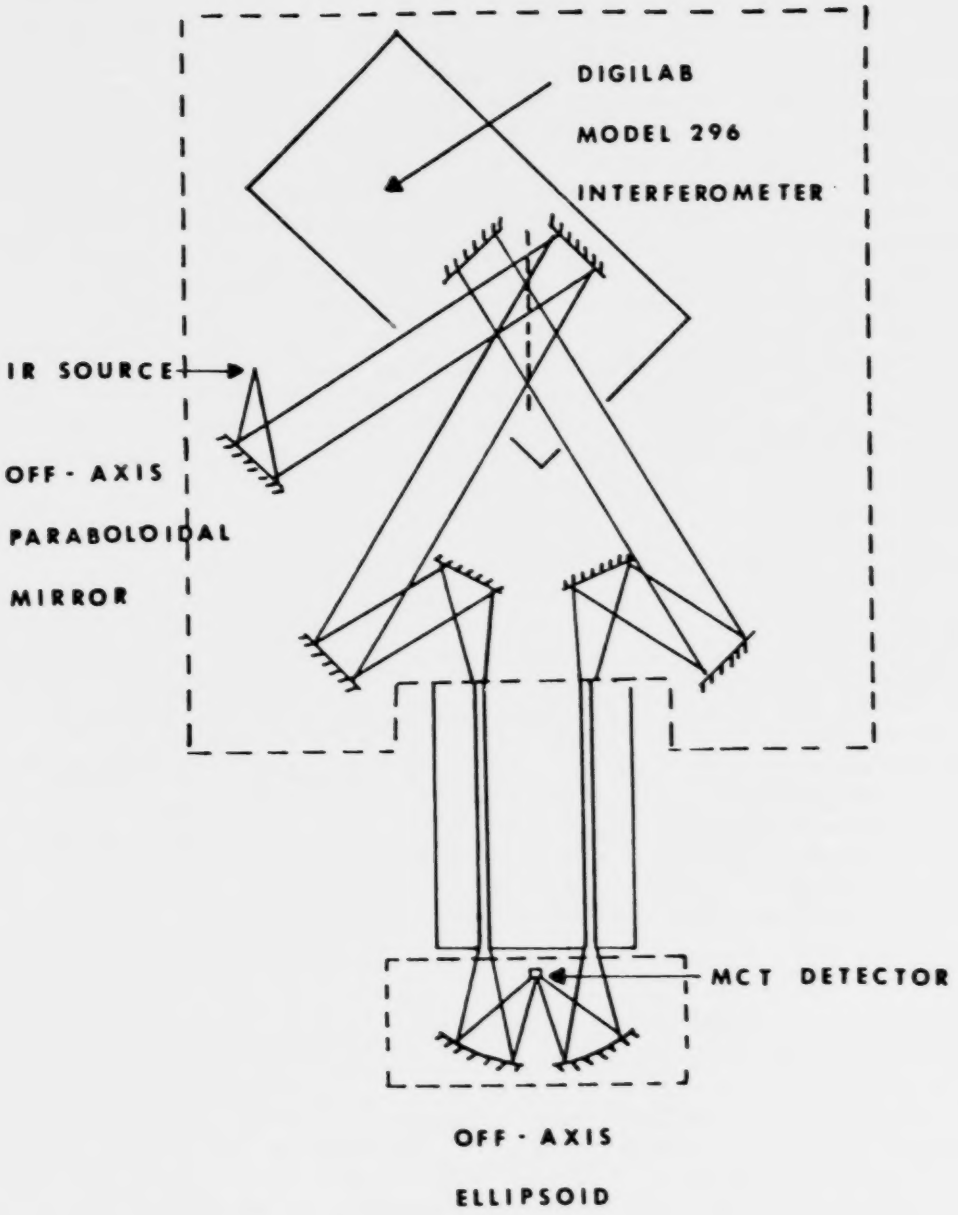

Figure 7. Optical layout for GC-IR using dual-beam FT-IR optics.

PRACTICAL PERFORMANCE

Nulling Ratio and Baseline Compensation

With no sample present in either beam it was never possible to completely null the interferogram. At the minimum amplitude of the dual-beam signal, the transform of this interferogram did not have the same profile as the single-beam spectrum, which would be the case if the incomplete null was due to optical misalignment. Apparently much of the spectral structure is due to species present on the surface of the germanium beamsplitter next to the compensator plate. Similar bands had been observed previously during earlier work using a low resolution rapid-scanning interferometer (9,10). To eliminate the effect of these bands and to create a flat baseline, a dual-beam interferogram was measured with no sample in either beam, and both this interferogram and its transform were stored. After the dual-beam interferogram of the sample was measured, baseline compensation was achieved by either subtracting the background interferogram from the sample interferogram or subtracting the background spectrum from the sample spectrum. For certain measurements the former procedure gave the flattest baseline while for others the latter procedure gave the superior results. We were unable to develop any rationale to forecast which procedure gave better background compensation.

Transmittance Spectra

That spectra corresponding to conventionally measured transmittance spectra can be calculated from dual-beam spectra is illustrated in Figure 8. In this series of spectra, which were measured using a TGS detector, A shows a dual-beam spectrum of a 50 μm film of polyethylene held in beam A, B shows a dual-beam background spectrum, and C shows the result of subtracting B from A. D is the single-beam background spectrum and E is the result of dividing spectrum C by -D and subtracting the result from unity. A comparison transmittance spectrum of polyethylene measured in the conventional fashion by ratioing a single-beam sample spectrum by a single-beam reference spectrum is shown in F. It can be seen that the sharp band at 720 cm^{-1} is distorted. This distortion is apparently caused by a problem in phase correcting the dual-beam interferogram; this problem will be discussed later in this section. However spectra E and F are otherwise identical.

S/N Advantage

The advantage, in terms of S/N, of the dual-beam system with an MCT detector over a conventional single-beam system with a TGS detector is illustrated in Figure 9 by spectra of a 0.01% solution of anisole in CCl_4 contained in a cell of 93 μm pathlength. For the dual-beam measurement, a variable pathlength cell containing pure CCl_4 was held in the reference beam, and its pathlength was adjusted to minimize the signal at the detector. For the single-beam measurement, the single-beam spectrum of the solution was ratioed against a single-beam spectrum of pure CCl_4 held in the same cell. Both sets of spectra were measured with 10 scans for sample and reference spectra. The strongest band in this spectrum had an absorbance of 1.7×10^{-4}, and the noise equivalent absorbance was less than 1×10^{-5}. It is apparent

that with less than 1000 scans, bands with an absorbance of less than 10^{-6} could be observed. The performance of the dual-beam system below 2000 cm^{-1} is at least an order of magnitude better than any spectrometer known to the principal investigator for highly transmitting samples.

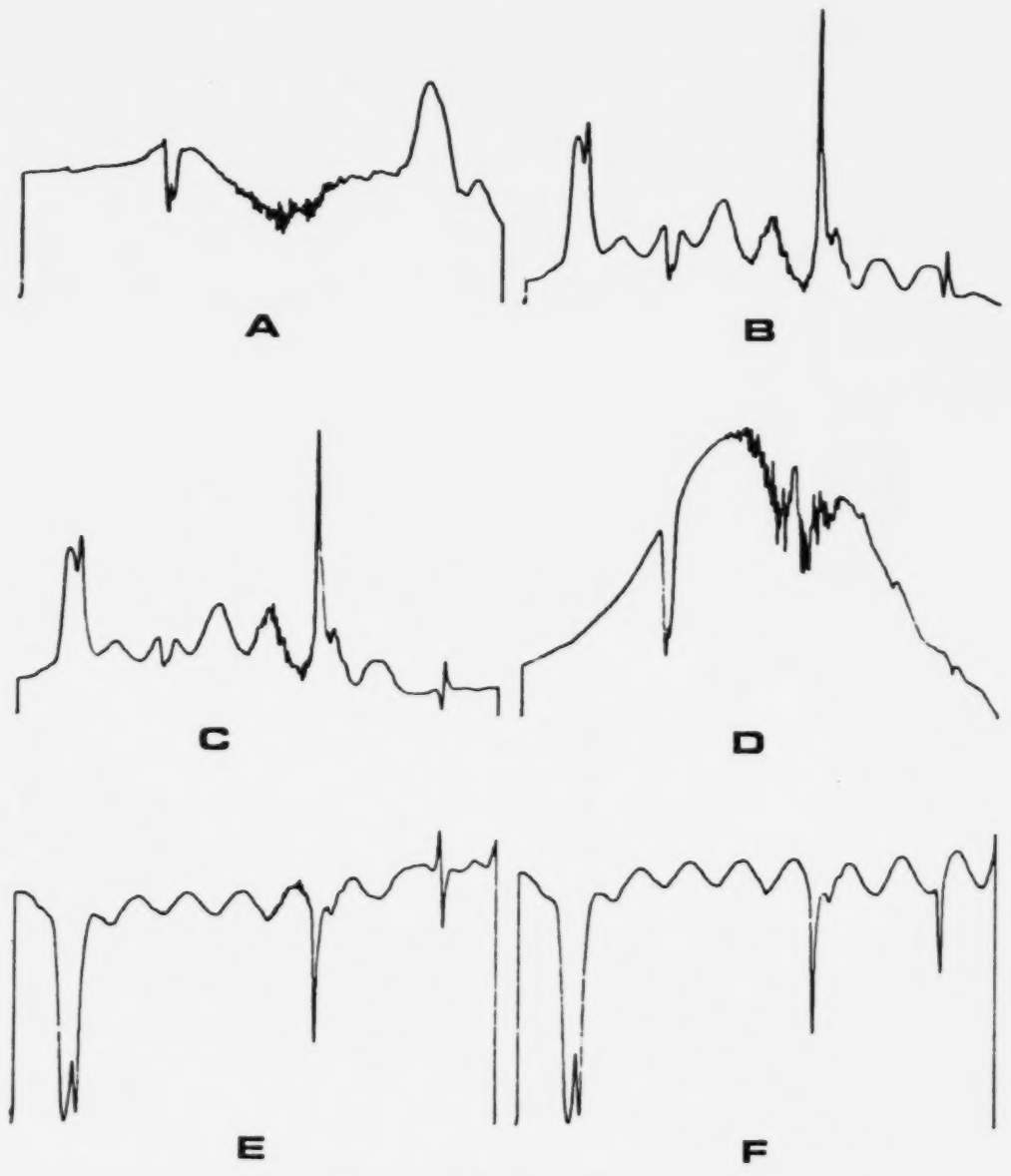

Figure 8. Calculation of a transmittance spectrum from spectra measured using a dual-beam FT-IR spectrometer (see text for details).

Phase Correction

The biggest problem encountered in this work was the generation of phase errors of the type mentioned earlier with reference to the distortion of the 720 cm^{-1} band in the polyethylene spectrum shown in Figure 8. Several approaches were taken to improve the accuracy of phase correction on our dual-beam FT-IR system, including the following:

a. extending the number of data points around the point of stationary phase used in the Mertz method of phase correction (13);

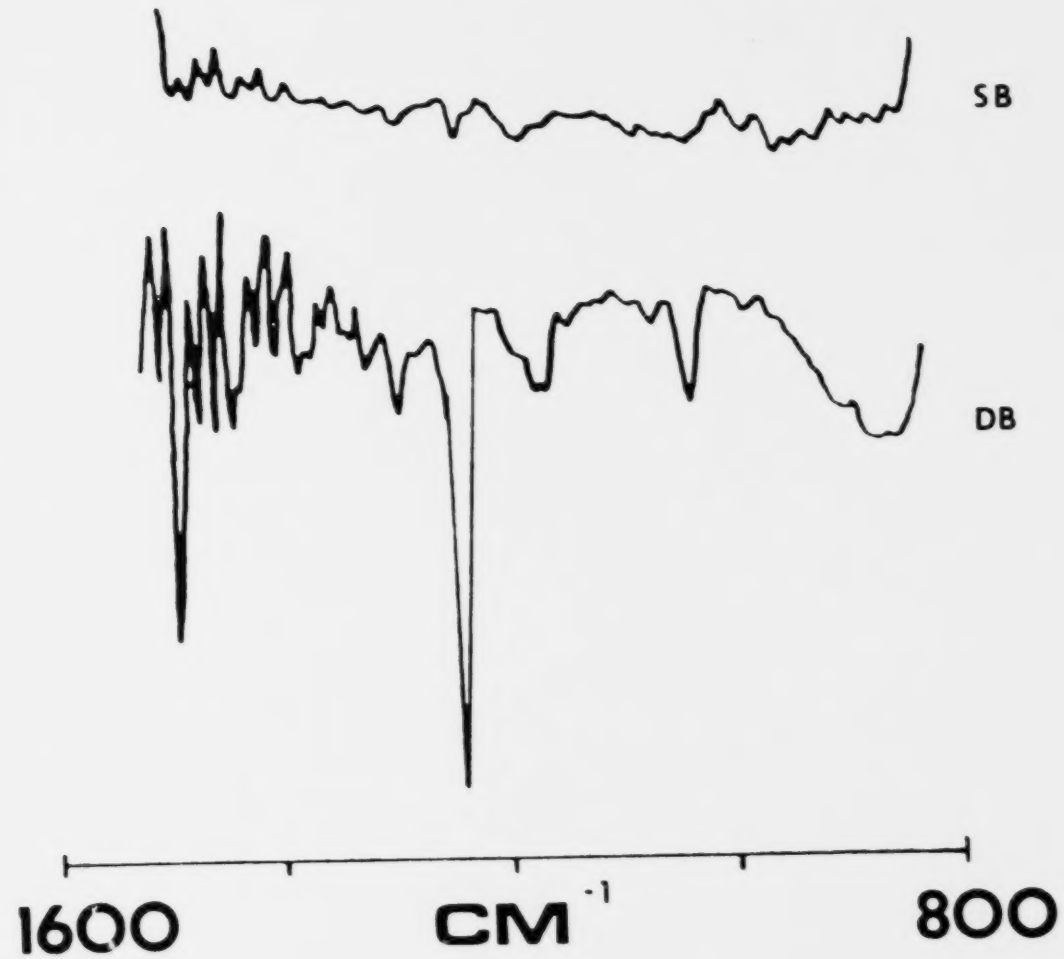

Figure 9. Spectra of 0.01% anisole in carbon tetrachloride at a pathlength of 96 μm measured using a single-beam (SB) FT-IR spectrometer with a TGS detector and a dual-beam (DB) FT-IR spectrometer with an MCT detector.

b. storing a phase spectrum calculated from a dual-beam interferogram of a screen with a spectrally uniform transmittance of about 10%, and using this array for subsequent transformations;

c. slightly misaligning the dual-beam optics to increase the amplitude of the interferogram near the point of stationary phase.

None of these approaches, however, gave significantly improved band symmetry.

The root of the problem was ultimately traced to an instability in our interferometer causing large variations in the shape of the measured interferogram from scan to scan. Although the magnitude of these variations was rather small for single-beam measurements (typically 1-2% near the point of stationary phase), the dual-beam interferograms could vary by as much as 50% in the same region, see Figures 10 and 11.

The problem appears to be confined to interferometers of the particular model being used for this project. The interferometer, which was purchased in 1973, shows no significant design changes from the first ones made by Block Engineering, and later by Digilab. When the moving mirror of these interferometers is retraced after each active scan, it is stopped by a foam rubber pad. An audible "thump" can be heard on impact. Apparently this sets the mirror into oscillation, and it has to be held stationary for a period of time (usually a little over a second) to allow these oscillations to die down before the next scan is initiated. Because the mirror has to be held stationary for such a long period of time, the duty cycle efficiency for signal-averaging is rather low - about 20% for measurements made at 8 cm^{-1} resolution on our instrument. Perhaps even more importantly for this project, even though the amplitude of the mirror oscillations does decrease during the period the mirror is held stationary, the mirror never seems to be completely stable, so that even when the duty cycle efficiency was intentionally decreased still further, significant scan to scan variations were still observed in the dual-beam interferograms.

The duty cycle efficiency of Digilab interferometers made after 1975 was improved by changing the way in which the moving mirror is retraced after each scan. As the mirror approaches the end of its retrace, its position is sensed photoelectrically, and a retardation pulse is applied to the coil of the drive transducer of large enough magnitude to halt the mirror and start it moving forward. After this point, the mirror motion is controlled by the same triangular ramp voltage as in the earlier interferometers. In this way the duty cycle for 8 cm^{-1} measurements has been increased from 20% to 90%. Thus four times as many scans can be taken per unit time, and the resultant spectra have a S/N which is twice as great as spectra measured in the same time on earlier interferometers.

Because this new fast retrace mechanism does not involve halting the mirror by a mechanical stop, there is less cause for it to be set into oscillation when it retraces; we therefore believed that the dual-beam stability should be much better if a new interferometer were used. We

Figure 10. Reproducibility of interferograms.
A. Interferograms measured on a dual-beam FT-IR spectrometer.
B. Interferograms measured on a single-beam FT-IR spectrometer.

Figure 11. Reproducibility of dual-beam FT-IR spectra of 0.1% anisole in carbon tetrachloride in a 96 μm cell. Each spectrum was computed from a single-scan interferogram. The effect of phase correction errors is readily apparent.

tested this hypothesis by driving our dual-beam optical bench to the Digilab facility at Cambridge, MA, and found that indeed the scan-to-scan variation in dual-beam interferograms was less than 2%. Were a new interferometer used for dual-beam measurements, the phase correction problem will be much alleviated. The improved photometric accuracy might even result in a further improvement in S/N, so that it may be possible to detect bands with an absorbance significantly less than 10^{-6} in a reasonable measurement time.

Detector Linearity

The actual sensitivity improvement of dual-beam FT-IR using an MCT detector compared with single-beam FT-IR using a TGS detector for measurements using typical sources at full throughput is of the order of factor of ten. Our initial calculations using manufacturer's quoted D* data suggested the improvement should have been about twice as good as it was found to be. However, when the intensity of the collimated input beam was attenuated using screens of various transmittance values the improvement factor was found to increase. This is presumed to be due to saturation of the detector at high photon flux. The response of the TGS detector increases linearly with photon flux, but the response of the MCT detector only increases with (approximately) the square root of photon flux, see Figure 12.

In spite of this factor operating against the use of dual-beam FT-IR, if the S/N of single-beam interferograms measured with a TGS detector is close to the digitization noise limit (which, in practice, it usually is) and one desires to increase the sensitivity through the use of an MCT detector, the largest advantage of dual-beam FT-IR compared to single-beam FT-IR is found at the highest photon flux. With no sample or accessory attenuating the beam, dual-beam FT-IR with an MCT detector gives about an order of magnitude improvement over the corresponding single-beam measurement. For GC-FT-IR measurements, the light-pipe usually attenuates at least 60% of the incident radiation, so that dual-beam FT-IR gives less than an order of magnitude advantage compared with the corresponding single-beam measurement with an MCT detector, but the advantage is still appreciable. This topic is discussed in greater detail in the next section.

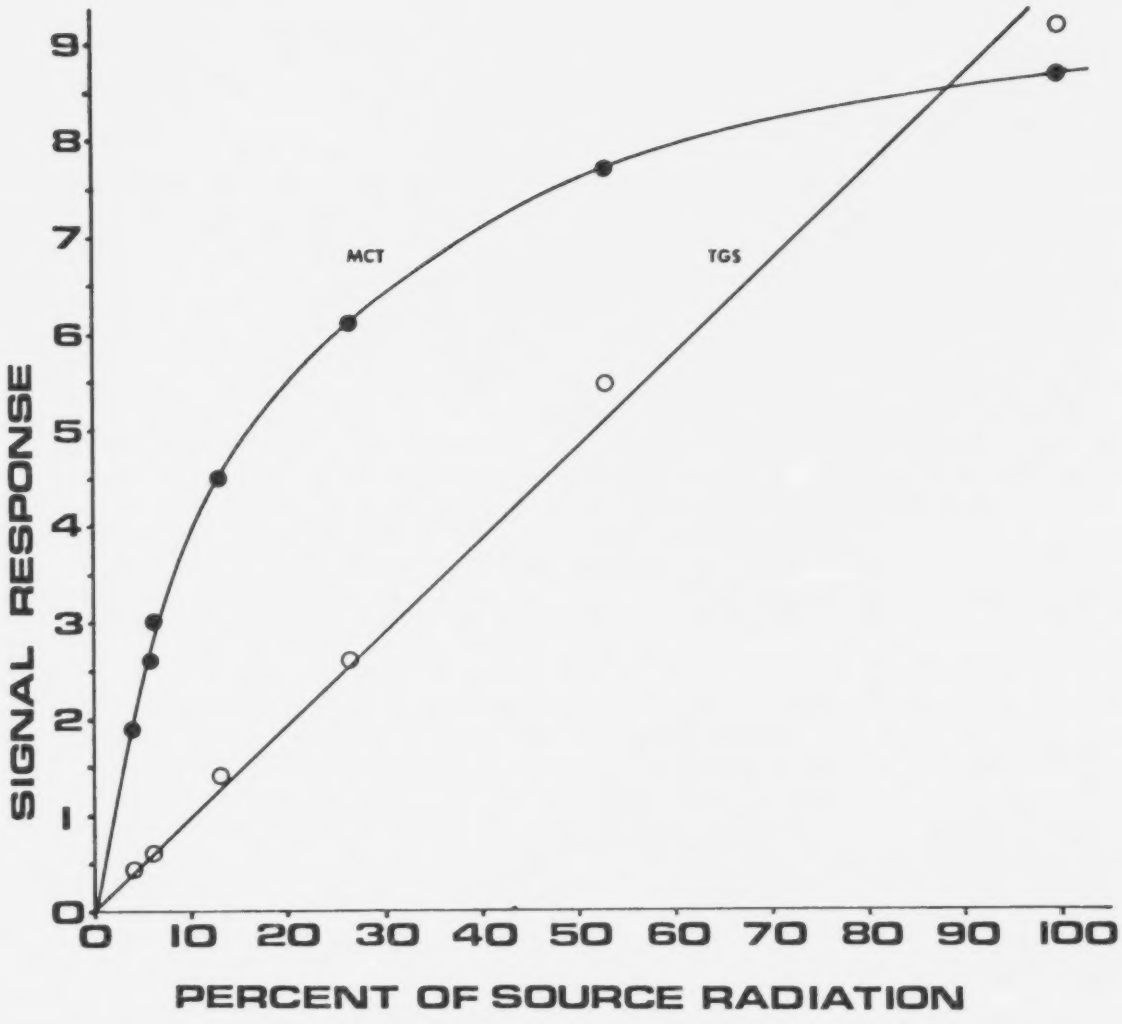

Figure 12. Linearity of the response of the MCT and TGS detectors at high photon flux. The scale for the TGS detector has been expanded by a factor of ten relative to the scale for the MCT detector.

SECTION 6

GAS CHROMATOGRAPHY AND FT-IR SPECTROSCOPY

GC-FT-IR SYSTEMS FOR PACKED GC COLUMNS

The first measurements in this project were optimized for gas chromatography performed using 1/8-in. o.d. packed columns. Typical peak half-widths for compounds separated in this way are 4 ml or greater. To study these peaks, we purchased and interfaced equipment consisting of two parallel light-pipes, each of which was 30 cm long and 4 mm square in cross-section (4.8 ml volume), held 6 inches apart in an oven assembly (Norcon Instruments, S. Norwalk, CT). Our first optical arrangement using these light-pipes was strictly a single-beam FT-IR system using only one of the light-pipes. The system was designed primarily for use with a TGS detector since the transmittance of these light-pipes was so large (approximately 30%) that interferograms measured using an MCT detector would have been digitization noise limited. The performance of this system was described in a previous E.P.A. report from this laboratory (14). Identifiable spectra of microgram quantities of components could be measured without trapping the samples, and a few bands of submicrogram quantities of strongly absorbing compounds could be observed. Later single-beam and dual-beam work (15) was performed using the optical system shown in Figure 7.

Comparative GC-FT-IR detection limits for spectra measured on-the-fly using a TGS detector (single-beam) and an MCT detector (single-beam and dual-beam) are summarized in Table 1. The single-beam measurements were taken on the dual-beam optical system with the reflected beam blocked, so that these data are directly comparable. As forecast in the previous section, it was found that dual-beam GC-FT-IR spectra measured with an MCT detector are slightly more than an order of magnitude more sensitive than measurements taken on the single-beam system with a TGS detector, and about a factor of four better than measurements taken on the single-beam system with an MCT detector.

The detection limits obtained were controlled not only by the type of optical system being used but also by the nature of the samples and the efficiency of the chromatography. Strongly polar, volatile compounds, such as anisole, could be detected at very low quantities (less than 100 ng), whereas less polar, less volatile compounds, such as the chlorinated pesticides, presented far more of a problem and often required an order of magnitude more sample to be injected into the chromatograph to yield an identifiable spectrum.

TABLE 1

Experimental detection limits for a group of organic compounds in the dual-beam and single-beam configuration

Compound	Detection limits (μg)		
	Single-beam		Dual-beam
	(TGS)	(MCT)	(MCT)
Anisole	0.8	0.20	0.050
Chlorobenzene	1.	0.25	0.075
Diethyl Malonate	2.	0.45	0.100
Acetonitrile	5.	1.5	0.400
Benzonitrile	10.	2.5	0.750
Aldrin	5.	1.5	0.400
Perthane	10.	2.5	0.750
p,p'-DDT	6.	1.5	0.400
Heptachlor	8.	2.0	0.600

All the detection limits in Table 1 refer to the amount of sample injected into the chromatograph. It should be recognized that after elution from the column the sample was passed into a 9:1 effluent splitter with 10% passing to a flame ionization detector (FID) and the remaining 90% passing to the light-pipe. In view of the width of the GC peak, not all of this fraction is present in the light-pipe at all moments during the measurement. Thus we believe the detection limits in Table 1 to be realistically achievable in practical situations. It may be noted that it has been a practice of several people working in the field of GC-FT-IR in the past not to use an effluent splitter and then only to quote the maximum amount of sample in the light-pipe rather than the total sample injected into the chromatograph.

IDENTIFICATION OF WATER POLLUTANTS BY GC-FT-IR

The identification of parts-per-million (ppm) and parts-per-billion (ppb) amounts of organic compounds in water is an important environmental problem. We have studied the feasibility of applying GC-FT-IR to assist in identifying organics at this level after sorbing them onto neutral macroreticular polystyrene resins and eluting them off the column using polar solvents (16-18). Table 2 shows the percent recovery from 20-50 mesh XAD-2 resin (Malinkrodt Chemical Co., St. Louis, MO) for solutions of chlorinated pesticides in distilled water at a level of 50 ppb. The water flow rate for most of these measurements was 20 ml/minute and 25 liters of this solution was passed through the resin. When all the solution had been passed through the column, the resin was allowed to dry. 100 ml of the eluting solvent was then passed through the column at a flow rate of 2 ml/minute. This solution was then evaporated down to a volume of 1 ml, and 10 μl aliquots were injected into the chromatograph.

Of the three eluting solvents tested, diethyl ether gave the best percentage recovery for each pesticide. However, with a water flow rate of

TABLE 2

Effect of varying the eluting solvent and the water flow rate on the recovery of some chlorinated pesticides using 20-50 mesh XAD-2 resin.

Pesticide	Percent Recovery			
	Acetonitrile (20 ml/min)	Hexane (20 ml/min)	Diethyl Ether (20 ml/min)	Diethyl Ether (100 ml/min)
Aldrin	50	43	94	83
Lindane	73	69	100	88
DDD	78	74	93	79
p,p'-DDT	69	72	93	80
Perthane	85	71	96	85
Dieldrin	89	65	95	83
Heptachlor	76	59	87	68
Hexachlorobenzene	83	65	89	78

20 ml/minute, it takes 20 hours to elute a 25 liter sample. The effect of increasing the flow rate above 20 ml/minute to 100 ml/minute decreased the percentage recovery slightly (typically to about 80%), but reduces the time required to elute 25 liters of water to about 4 hours.

Representative GC-FT-IR spectra of solutes recovered from aqueous solution in this way using the dual-beam GC-FT-IR configuration shown in Figure 7 are illustrated in Figures 13 and 14. Figure 13 shows the spectra of these solutes recovered from 25 liters of a solution spiked with 2 ppb of each compound. A maximum of 500 ng of each was injected into the chromatograph (assuming 100% recovery) with 10% of the effluent stream being split to the FID. Each of these three compounds was a relatively strong infrared absorber, so that these spectra represent the highest sensitivity which we have been able to achieve for recovered trace organics from water. Figure 14 shows the spectra of three far weaker infrared absorbers, chlorinated pesticides, recovered from 2 liters of a 50 ppb solution of each. The infrared spectra of the two hexachlorocyclohexane isomers are quite different, in contrast to their mass spectra which are almost identical. The acquisition of information in isomeric molecules is, of course, one of the main advantages of GC-IR over GC-MS.

The sensitivity of these GC-FT-IR measurements is still lower than state-of-the-art GC-MS systems, but the technique should still prove to be of considerable benefit for the study of trace organics in water. It should be possible to reduce the detection limits of trace organics in water by GC-FT-IR in three ways:

a. The volume of the extract from the column could certainly be reduced below 1 ml; reduction of the volume to 100 µl should reduce the detection limits by about an order of magnitude. In addition several efficient sample concentrators are now commercially available (at a price usually between $1000 and $2000). Budgetary limitations have so far prevented us from investigating

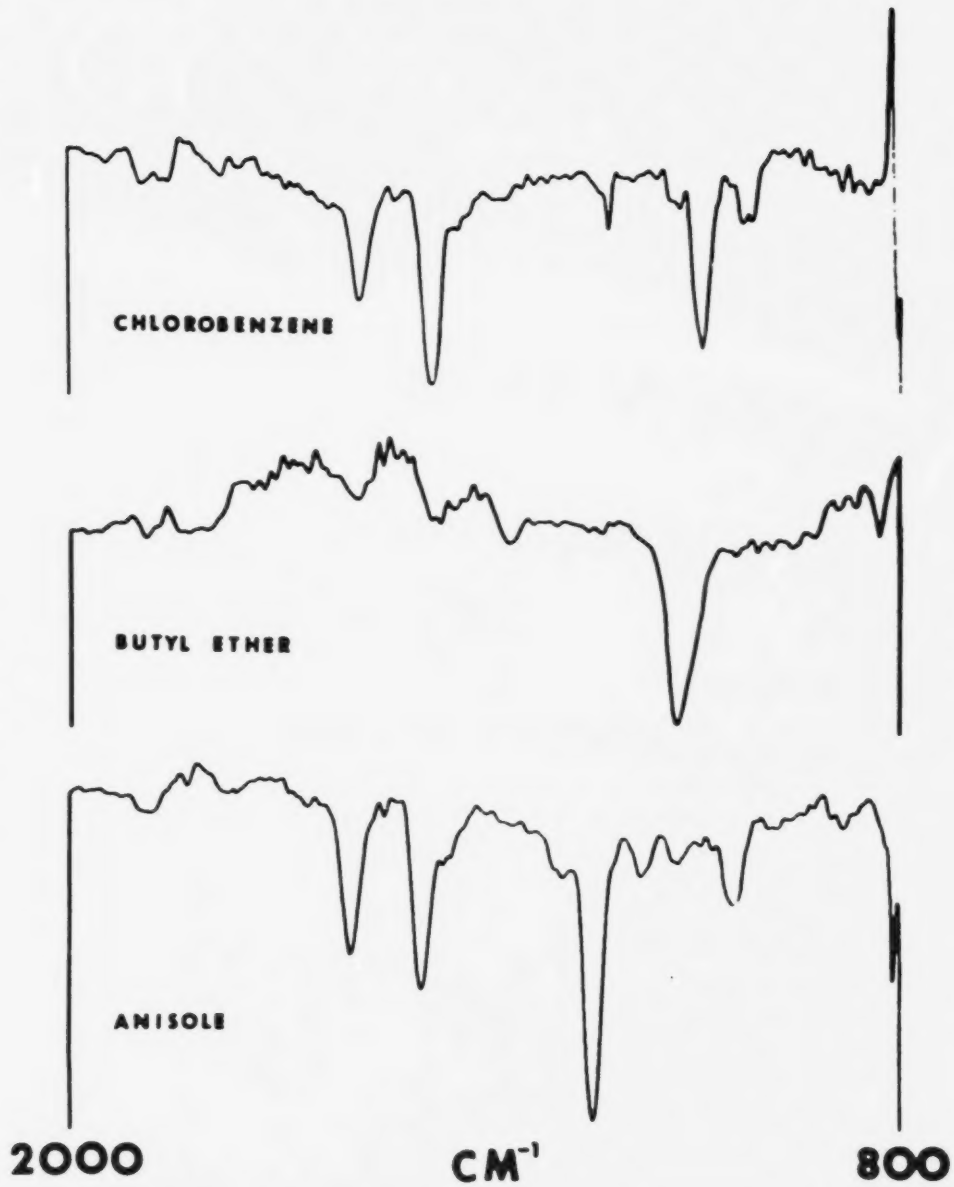

Figure 13. On-line GC-IR spectra of chlorobenzene, butyl ether and anisole recovered from 25 liters of a water solution containing 2 ppb of each component.

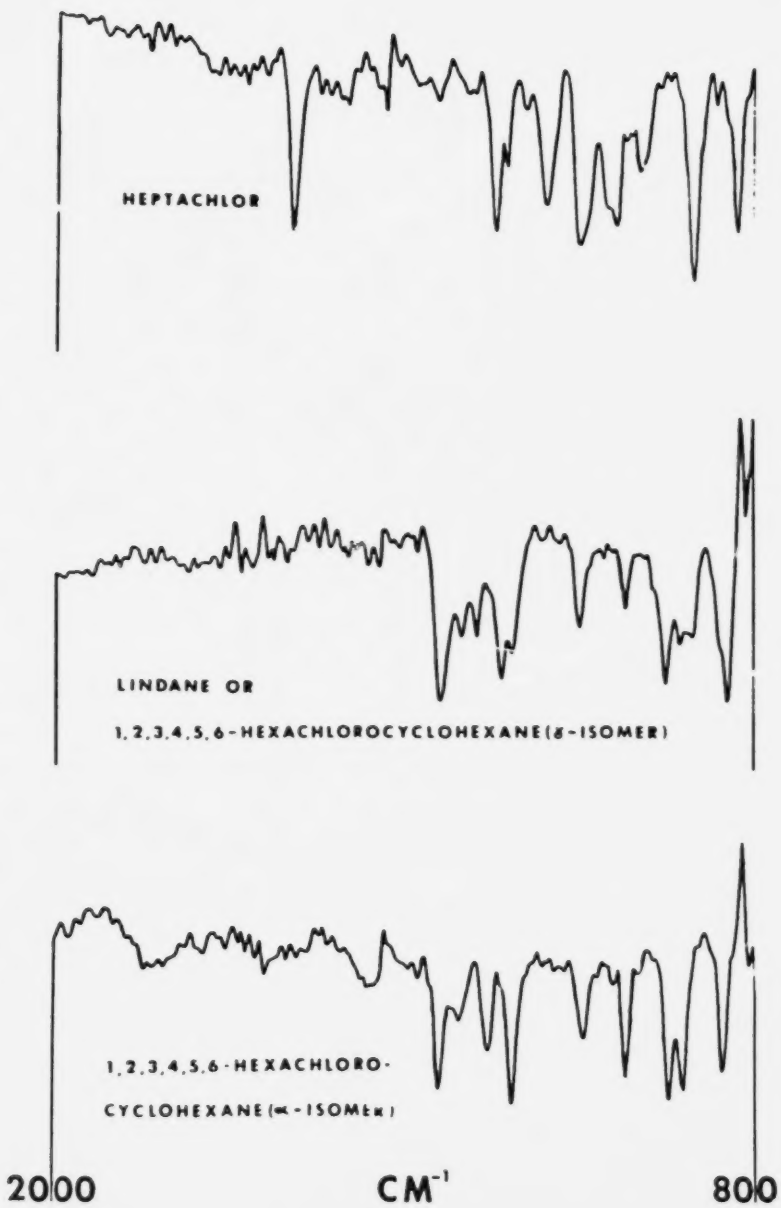

Figure 14. On-line GC-IR spectra of Heptachlor, and two isomers of 1,2,3,4,5,6-hexachlorocyclohexane recovered from 2 liters of a water solution containing 1 ppm of each component.

their application to this project.

b. We have demonstrated that the performance of the FT-IR spectrometer used for this project was not as good as that of contemporary systems (4). Figure 15 shows a comparison of the performance of our spectrometer to that of a new instrument made to the same nominal specifications by the same manufacturer for spectra measured by signal-averaging the same number of scans. Upgrading both the optics and the electronics of our spectrometer will certainly improve its performance, especially at high wavenumber. Installation of the fast retrace mechanism, discussed in Section 5, will not only increase the duty-cycle efficiency by approximately a factor of four for low resolution measurements, but could also yield a substantial improvement in the sensitivity of dual-beam GC-FT-IR measurements by improving the reproducibility of the interferograms. Again, because of budgetary limitations we were not able to update our spectrometer and consequently the detection limits that could be attained ultimately can only be estimated. An improvement of at least a factor of four would have to be achieved since merely improving the duty cycle efficiency would double the S/N attainable in a given time. The remaining factor of two improvement is very conservative in light of the data in Figure 15.

c. It is also possible to improve the chromatography and decrease the detection limits of GC-FT-IR from our early results summarized in Table 1. We have modified our chromatograph for capillary columns and the results are summarized below.

GC-FT-IR WITH CAPILLARY COLUMNS

We have shown (1) that decreased detection limits for GC-FT-IR should be achieved by switching from packed columns to capillary columns. The most popular type of capillary column, the wall-coated open-tubular (WCOT) column, does not have a high enough sample capacity to be useful for GC-FT-IR measurements. However support-coated open-tubular (SCOT) columns, while not achieving quite the resolution of WCOT columns, have a substantially greater sample capacity, and we have developed a GC-FT-IR system which is specifically designed for identifying samples separated on SCOT columns. The injection port, splitter assembly and transfer lines, and light-pipes were all modified for this work.

Injection Port

The on-column injector previously installed on the Model 3920 gas chromatograph (Perkin-Elmer Corporation, Norwalk, CT) which was previously used with the packed columns was replaced by a "splitless injector" (Scientific Glass Engineering, Dallas, TX). This device works on the same principles as the original device developed by Grob and Grob (19-21), and differs radically from conventional injectors used for capillary column chromatography. On conventional injectors, a splitter typically allows 1%

of the injected sample to pass onto the column; for a 1 µl injection, therefore, only 10 nl passes onto the column to avoid overloading by any component (especially the solvent). With a splitless injector, the whole sample (again typically 1 µl) is injected onto the column and can be treated in several ways:

a. If all the peaks are well separated from the solvent peak, elution can occur without interruption.

b. If there are several peaks which elute close to the solvent peak, the injected sample is subjected to the flow of carrier gas for about 20 seconds, to ensure that all the sample is on the column. A valve is then opened for about 40 seconds, and the back pressure of carrier gas in the column forces the more volatile components (especially the solvent) back down the column while the less volatile components are retained. After the solvent has been largely eliminated in this way, but before the other components have a chance to be lost, the valve is closed and the elution proceeds. Not only is the solvent interference reduced in this way, but the capacity of the column for samples of only slightly greater volatility than the solvent is increased. In addition, while the valve is open all the components tend to be reconcentrated

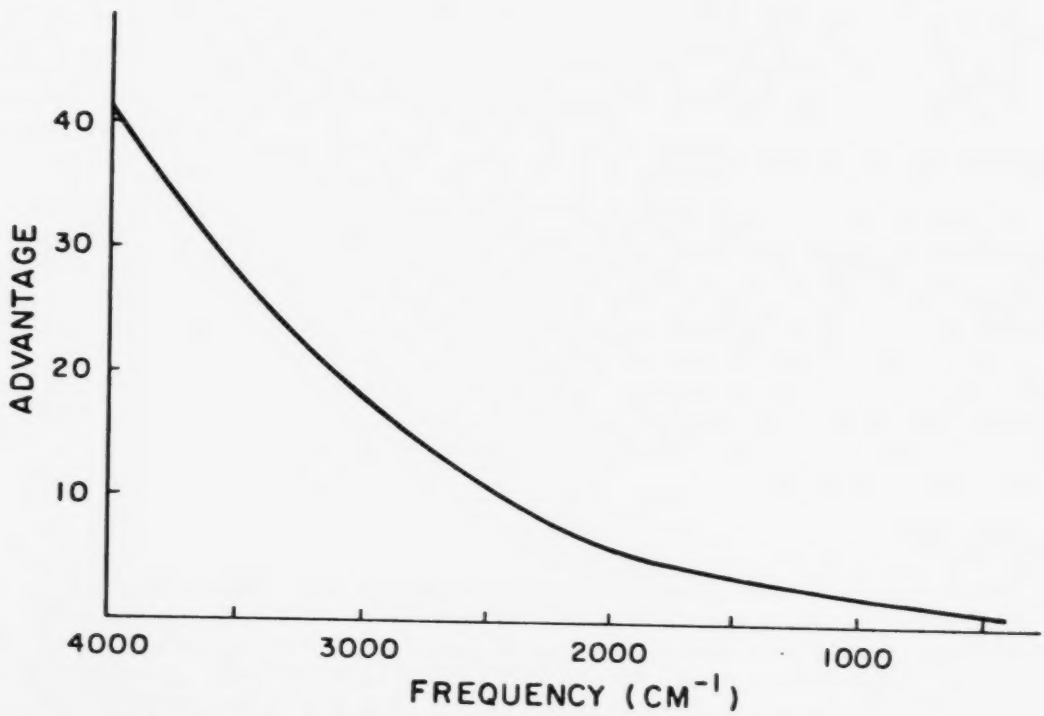

Figure 15. Improvement in S/N of a new FT-IR spectrometer over the instrument used in this study.

near the head of the column (19-21) and the resolution of the chromatogram is increased slightly in a way which is analogous to the first step of programmed multiple development thin-layer chromatography, which is described in Section 8.

c. If there is appreciable bleeding from the septum, the valve need not be fully closed and a small fraction of the carrier gas is not passed through the column but rather is vented to the atmosphere. This proceedure extends the useful life of SCOT columns considerably and we use it all the time (after the process described in b. above, if necessary).

Columns

Both stainless steel- and glass-walled SCOT columns were used in this work; all columns were 50 m in length. The glass columns gave the most symmetrical peaks but were far more difficult to interchange on our chromatograph.

Detector and Splitter Assembly

In our earlier work with packed columns, the effluent from the column was first passed through the thermal conductivity detector (TCD) which was originally installed on our chromatograph. The effluent was then split, with approximately 10% of the gas passing to a flame ionization detector (FID) (Gow-Mac Instrument Company, Madison, N.J.) and 90% passing to the light-pipe. The GC resolution was almost certainly degraded by the rather large dead volume of the TCD. In addition, the performance of the FID was degraded by the rather low flow rate of carrier gas, which was only 10% of the flow rate through the column. The flow rate through SCOT columns is typically about 2ml, and we were unable to achieve good response from the FID when SCOT columns were directly substituted for packed columns.

The problems were overcome first by decreasing the dead volume introduced by the TCD by completely removing the TCD from the chromatograph and installing the effluent splitter close to the end of the column in a well insulated part of the chromatograph. The FID was then mounted close to the effluent splitter in the same part of the chromatograph. A T-junction was installed between the splitter and the FID into which make-up helium could be passed to improve the performance of the detector. Increasing the flow rate of carrier gas through the detector to 20 ml/minute was beneficial when packed columns were used for the chromatography and absolutely essential for SCOT columns.

Transfer Line

The dead volume between the splitter and the light-pipe was decreased by substituting the 1/8-in. o.d. tubing used with packed columns by 1/16-in. tubing.

Light-Pipes

The Norcon light-pipes used in our earlier work were too large for use with SCOT columns. We therefore prepared our own light-pipes (optimized for SCOT columns) using the method of Azarraga (22). Both glass and glass-lined stainless steel tubing (Scientific Glass Engineering, Dallas, TX) were coated by first scrupulously cleaning them and then coating them with a layer of "Liquid Bright Gold" paint (Engelhard Industries, East Newark, N.J.). After the paint, which is a gold complex, was deposited as a uniform layer, the complex was decomposed by heating the tube in an atmosphere of air, leaving a uniform layer of gold on the interior of the tube. We first used a design for the light-pipe also sent to us by Azarraga (22) but found the flow characteristics of this light-pipe caused excessive tailing of sample peaks. We therefore developed a new design in which the gas from the chromatograph was fed directly into the light-pipe by means of a 1/16-in. o.d. tube. The end of this tube was manually filed down until the wall thickness was very small. A groove was then cut into the ends of the light-pipe and the filed end of the tubing was sealed in place. A NaCl window was sealed onto the end of the light-pipe with the same adhesive. Only glass tubes could be prepared in this way, since we could find no way of preventing the thin glass layer of glass-lined stainless steel tubing from cracking.

Preliminary results with this system have, frankly, been disappointing. The problems have largely been associated with the chromatograph rather than with the spectrometer. If a flame ionization detector is used to measure the chromatogram, an effluent splitter must be used to allow most of the effluent to pass through the light-pipe rather than the destructive GC detector. With the 10:1 effluent splitter we were using, the chromatograms measured from SCOT columns were very unreproducible, and we were never certain how much, if any, samples was passing through the light-pipe. We believe that if the FID is replaced by a non-destructive photoionization detector, thereby eliminating the need for an effluent splitter, more reproducible results will be obtained. We are currently in the process of obtaining such a detector, and we confidently predict that when all the operating parameters are optimized, detection limits for GC-FT-IR using SCOT columns will be reduced below 10 ng.

SECTION 7

LIQUID CHROMATOGRAPHY AND FT-IR SPECTROSCOPY

USE OF FLOW-THROUGH CELLS

The problems associated with the on-line measurement of the infrared spectra of samples as they elute from a liquid chromatograph (LC-IR) are even more severe than those associated with GC-IR. The principal difference between GC-IR and LC-IR lies in the fact that for GC-IR the mobile phase is infrared transparent, so that light-pipes of any pathlength can be used without interference through absorption by the carrier gas, whereas for LC-IR all solvents used as the mobile phase absorb strongly at certain wavenumbers and less strongly at all others. In particular, for the polar solvents used in reverse-phase high performance liquid chromatography (HPLC) absorption by the mobile phase is sufficient to eliminate wide regions of the spectrum for study, even for pathlengths of 100 μm or less.

In single-beam GC-FT-IR it is possible to reduce the background signal at all wavenumbers using a long, narrow light-pipe with substantial reflection losses, and to maintain a high S/N by using an MCT detector. The loss in signal caused by reflection losses is approximately uniform across the spectrum, and the response profile of an MCT detector in a rapid-scanning FT-IR spectrometer above 800 cm^{-1} is not significantly different from that of a TGS detector. Thus by suitably designing the dimensions of the light-pipe so that detector noise exceeds digitization noise, substantial gain can be made in single-beam GC-FT-IR when an MCT detector is used.

On the other hand, in a single-beam LC-IR experiment, if the pathlength of the cell is increased, the transmittance at regions where there are no absorption bands remains very high, but in the regions of strong solvent bands the transmittance may change drastically. For example, if a band has a peak transmittance of 10% for a certain cell thickness, it will have a transmittance of only 1% if the pathlength is doubled. If a weak solute band absorbs at the same wavenumber, doubling the pathlength will double the absorbance, and to a good approximation the absorptance, of the band so that the S/N of that band would be <u>reduced</u> by a factor of five on doubling the pathlength of the cell. It can be readily shown that the optimum value for the S/N of solute bands is achieved when the solvent transmittance is 1/e or 36.8%. Of course no solvent has a uniform transmittance, and the decision has to be made as to the best pathlength to permit a "reasonably large" proportion of the spectrum to be observed with an acceptable S/N after solvent compensation.

The range of acceptable solvent transmittance which will permit solute spectra to be measured at an acceptable S/N may be estimated by calculating the product of the solute absorbance and solvent transmittance and plotting the result against solvent transmittance, see Figure 16. This graph shows that the optimum sensitivity is found when the solvent transmittance is 36.8%, as expected, and that spectra may be measured within 75% of the optimum sensitivity if the solvent transmittance is between 65% and 14%. The corresponding 50% points are between 79% and 7% solvent transmittance. Thus the pathlength of the cell should be chosen so that the transmittance of the solvent in the fingerprint region of the spectrum is between about 80% and 10%. If the transmittance falls below about 2.5%, i.e. in the region of strong solvent bands, essentially all solute information is lost.

For many organic solvents (with the exception of those containing a nitrile group), the transmittance between 2700 and 1800 cm^{-1} exceeds 90% when the transmittance in the fingerprint region is optimized, but since so few solutes have interesting absorption bands in this region, this high transmittance cannot be used to increase analytical sensitivity (again, with the exception of nitriles). The large amount of transmitted energy in this region has the disadvantage of increasing the dynamic range problem for single-beam LC-FT-IR measurements made with an MCT detector. If the optimum pathlength for flow-through cells is achieved for measurements made in the fingerprint region of the spectrum, it is apparent that high sensitivity LC-FT-IR must use dual-beam techniques to attain the lowest possible detection limits.

For many solvents used in HPLC, a pathlength of approximately 100 μm appears to be optimum, with only a few spectral regions where the transmittance is less than 10%. However even for bands of intermediate strength, $10\% < T < 90\%$, the problem of accurate solvent compensation to leave a flat baseline for the solute spectrum may be quite severe. It should also be noted that the absorbance of solutes eluting from an HPLC is going to be very small if a 100 μm cell is used, e.g. the absorbance of the strongest bands in a 1% solution of the pesticide DDD in hexane at this pathlength is about 0.1. Even though a peak concentration of 10 μg/ml of solutes eluting from an HPLC would be considered quite typical by most chromatographers, the maximum absorbance of DDD measured under these conditions would be 10^{-4}, or T = 99.98%. This is a rather low concentration to give detectable bands by single-beam FT-IR and if the bands absorb close to solvent bands absorbing more than 70% detectable bands could not be measured in less than one minute's data acquisition time. The need for dual-beam FT-IR to increase sensitivity again becomes apparent.

The optical arrangement we used for dual-beam LC-FT-IR is the same as that shown in Figure 6, with a flow-through cell in the transmitted beam and a variable pathlength reference cell in the reflected beam. The flow-through cell was 4 mm in diameter and 93 μm in pathlength, so that its volume was 1.2 μl. Since the half-width of HPLC peaks separated using columns packed with 10 μm diameter particles is typically 500 μl-1 ml, it can be shown that less than 1% of the sample is present in the cell at any moment during the measurement. The volume of the cell could be increased by increasing the diameter of the cell, but since the diameter of the beam at the sample focus is only 3 mm, no improvement in S/N would be noted. Increasing the path-

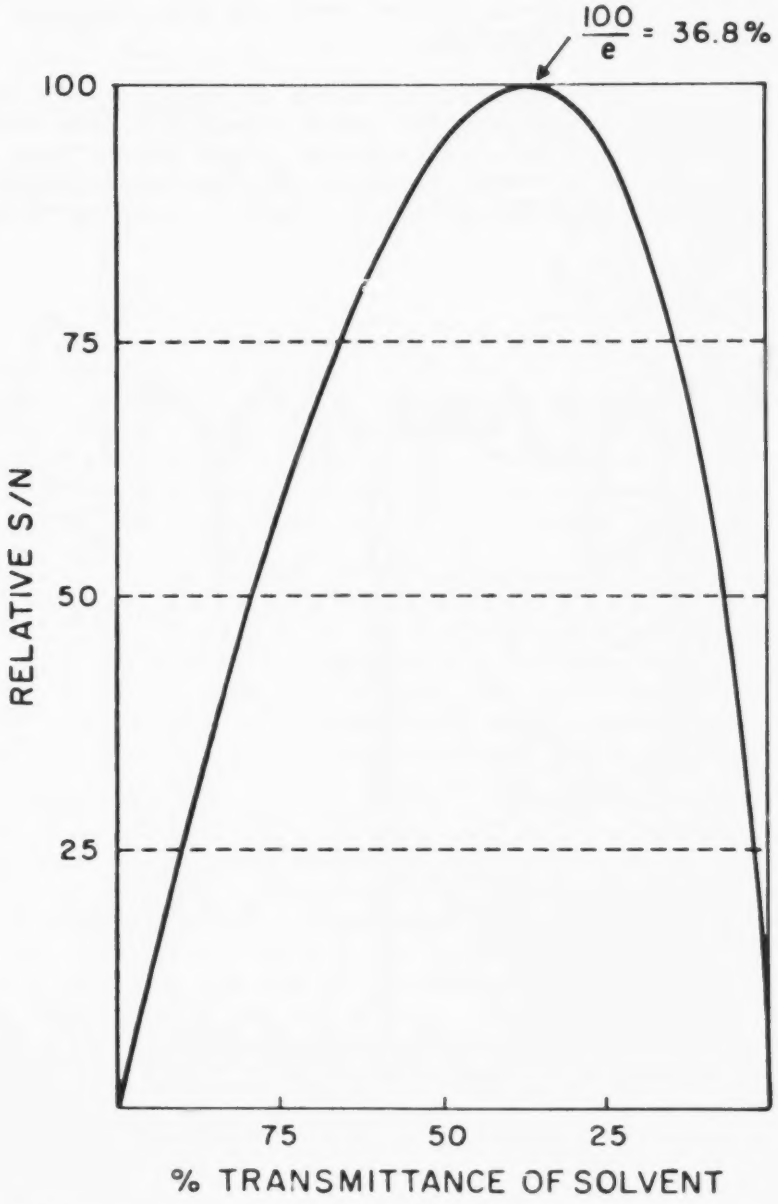

Figure 16. Ratio of the experimental to the optimal S/N for weak absorption bands as a function of the transmittance of the solvent. The sensitivity at any given wavenumber may be increased by varying the pathlength of the cell to yield a transmittance of 36.8% at that wavenumber.

length of the cell would increase the efficiency in regions where the solvent absorbs weakly, but would increase the number and extent of the regions which would be "blacked out" by strong solvent bands and also seriously increase the problems of solvent compensation.

As might be expected the detection limits of on-line LC-FT-IR measurements made using these optics were not spectacularly low. The best results were found with strongly polar, low molecular weight solutes such as anisole. For such samples, 100 µg of sample injected onto the chromatograph would yield a readily identifiable spectrum, see Figure 17, and detection limits of

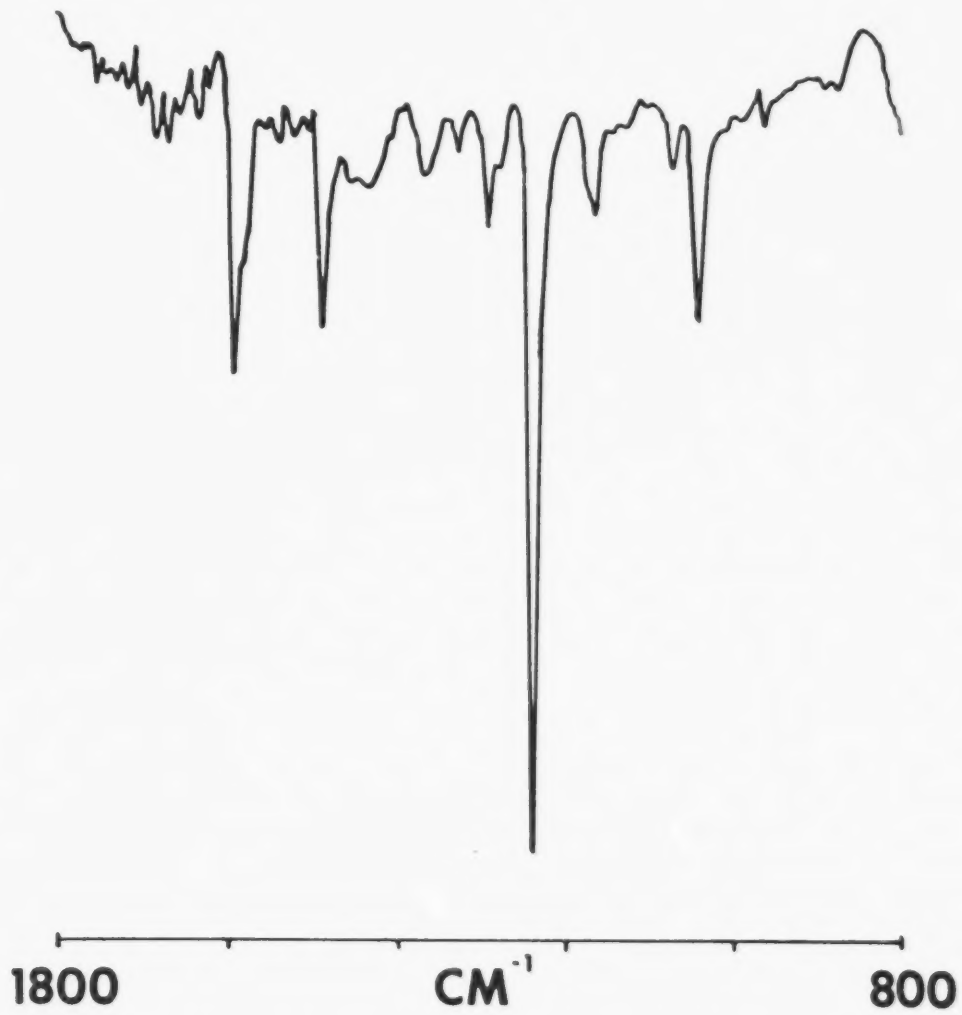

Figure 17. On-line LC-FT-IR spectrum of 100 µg of anisole, a strong absorber, injected into the chromatograph, eluted with hexane, and measured by dual-beam FT-IR spectrometry.

approximately 10 μg were obtained. However for less polar, more weakly absorbing materials, such as the chlorinated pesticides, detection limits about an order of magnitude greater than this were found, see Figure 18. To what extent the rather poor solvent compensation evident in this spectrum is caused by the instability of the interferometer cannot be judged. If this effect is the principal cause of baseline variation, it is quite possible that detection limits could be reduced considerably.

Figure 18. On-line LC-FT-IR spectrum of 150 μg of TDE, a weak absorber, eluted with hexane, and measured by dual-beam FT-IR spectrometry. A reference spectrum of TDE is shown above.

It is, of course, possible to increase the amount of solute present in the flow-through cell by increasing the volume of sample injected into the chromatograph. However we found that increasing the quantity of what is already enough solute to exceed the linear capacity of the column degraded the chromatographic resolution. Only for mixtures with very few components was increasing the volume injected found to be beneficial.

A further disadvantage of on-line LC-FT-IR measurements of the type described above is the fact that they can only be performed for isocratic separations. If gradient elution is required to improve the quality of the chromatogram, the problems of solvent compensation are so great that this method of LC-FT-IR becomes essentially useless.

All the above problems stem from the absorption of the incident radiation by the mobile phase. We have therefore studied various ways for performing continuous or semi-continuous LC-FT-IR measurements after removing the solvent in some fashion.

SOLVENT ELIMINATION TECHNIQUES

The first technique we studied was to evaporate the solution onto a heated moving metal ribbon so that the solvent evaporated and the solute was deposited on the ribbon. After removal of the solvent and the section of the ribbon on which the solute was deposited has moved into the infrared beam, the reflection-absorption spectrum of the solute could be measured. The problem with this idea is that, if the flow rate through the chromatograph is greater than about 500 $\mu l/min$, the area over which the solute is deposited is much greater than 1 cm^2, whereas the maximum sensitivity is found if the area is at least ten times smaller than this. We found that the best way to obtain the smallest deposition area was to spray the solution onto the ribbon so that much of the evaporation could take place before the solution came in contact with the ribbon. However even with this technique, the area over which the solute was deposited was so large that the detection limits were merely comparable to those found for the on-line experiments made with a flow-through cell.

Another method of obtaining low detection limits for solutes is to evaporate the solution on the surface of a micro attenuated total reflection (μ-ATR) crystal. By using small (0.5 or 1 mm thick) crystals of less than 1 cm^2 surface area, detection limits of less than 100 ng have been reported for off-line experiments using grating spectrophotometers (23). The usual procedure in measurements of this type is to take a rather large volume of the solution and evaporate off all but a few microliters of solvent by blowing air through the warm solution. The remaining concentrated solution is applied to the surface of the μ-ATR plate using a microsyringe and the remaining solvent is evaporated off before measuring the spectrum of the deposited solute. This two step procedure cannot be applied for semicontinuous LC-FT-IR, and the evaporation of solvent at rates around 1 ml/min onto a surface area less than 1 cm^2 again proved very difficult. Although spraying again improved the efficiency of the deposition, we were never able to achieve the desired rate of deposition of 1 ml/min, and this approach was also ultimately abandoned.

It is apparent that for semicontinuous LC-FT-IR, the requirements are quite difficult to achieve. To achieve the maximum FT-IR sensitivity the beam diameter for the measurement should be small (typically 2-4 mm) but the rate of evaporation of solvent should be fast, which implies that the solute will be deposited over a large surface area. In addition the spectrum should be measurable a short time after the peak has eluted from the chromatograph, preferably using multiple passes of the beam to enhance sensitivity. One technique in which these criteria appeared to be obeyed involves spraying the solution into a heated vertical light-pipe. For a 3 mm diameter light-pipe of length 28 cm the interior surface area on which the sample is deposited is 26.4 cm^2, while the area of the aperture is only 0.07 cm^2. We proposed that by suitably controlling the temperature of the light-pipe it would be possible to deposit all the solute on the interior of the light-pipe while evaporating the solvent and flushing it out of the tube by the spray propellant gas. A beam entering the light-pipe would be multiply reflected as it passed down the tube that the reflection absorption spectrum of the sample would be enhanced.

We believed that it should be possible to construct a device in which four light-pipes are placed in a carousel holder. Into one of these the solute from the first HPLC peak would be deposited. After this peak has eluted and been deposited, the carousel would be rotated through 90°, after which the second HPLC peak would be deposited while the interferogram of the first peak is measured. After the second peak has been deposited the carousel would again be rotated through 90°. The third HPLC peak would then be deposited, the second peak would be measured and the first peak is washed from the tube. This process would be continued, with the fourth position on the carousel being used for drying and heating the tubes in preparation for the following deposition step. The complete system would be controlled by monitoring the signal from the HPLC detector using a second derivative trigger of the type described in Section 4. The circuit we used for this purpose was quite functional; however ultimately we planned on replacing it with a microcomputer used in the same fashion as in our later work with diffuse reflectance spectrometry.

The preliminary experiments performed to test the feasibility of this device were performed using solutions of a single colored component at low concentration (0.1-100 ppm) flowing into the light-pipe at rates around 1 ml/min; the flow was generated by a peristaltic pump. The first results from this system were marred when it was found that the principal sample which was deposited on the light-pipe was the phthalate ester plasticizer from the tubing of the peristaltic pump, although it should be noted that UV-visible spectrophotometry showed that the sample is deposited with high efficiency at the same time as the plasticizer.

After changing all tubing to Teflon, we were surprized to find that it was not possible to obtain identifiable spectra from sample quantities smaller than 100 µg even though all the sample was shown spectrophotometrically to be present in the light-pipe. The reason for this low sensitivity became apparent at the end of a series of experiments designed to determine the distribution of the deposited sample down the light-pipe. By depositing a colored compound,

methylene blue, it was possible to determine the distribution as a function of spray propellant gas flow rate and the light-pipe temperature (or, more specifically, the voltage applied to the nichrome wire heater coil wrapped around the light-pipe). The quantity of methylene blue deposited at intervals of 1 cm down the light-pipe was determined by dipping the first centimeter of light-pipe into a known volume of methanol to dissolve all the dye in this interval, and determining the concentration spectrophotometrically. This process was repeated at 1 cm intervals up the tube. The variation of solute quantity down the light-pipe is illustrated in Figure 19. The optimum results should be found when the sample is most uniformly distributed down the light-pipe, but even with a flow rate of 4 cfm of nitrogen and a Variac setting of 4, it was still found not to be possible to measure identifiable spectra from less than 100μg of deposited sample.

Several different attempts were made to improve the sensitivity of this technique. Initially 3 mm diameter light-pipes were used whose transmittance was so high that a screen of 12% transmittance was placed in the beam to avoid digitization noise. When 200 μg of methylene blue was deposited and measured in this way, the maximum absorptance was only about 10%, see Figure 20.a. To increase the number of reflections down the light-pipe, the screen was removed and replaced by an "antiaperture" to remove the central 90% of the area of the collimated beam emerging from the interferometer and leave only the outer ring. Surprisingly no increase in the absorption band intensity was noted, see Figure 20.b.

Finally the optics were changed so that light-pipes of smaller diameter could be studied. The 3 inch focal-length off-axis paraboloid used to focus the collimated beam onto the 3 mm diameter light-pipes was replaced by a 1.5 inch focal-length off-axis paraboloidal mirror. The reduction in the size of the focus enabled 1.5 mm i.d. light-pipes to be used without significant vignetting losses. In view of the reduced diameter of the light-pipe and the increased beam half-angle an increase in the absorbance of the bands should have been noted, but again, in practice, no improvement over the earlier measurements was noted, see Figure 20.c.

In each of the spectra shown in Figure 20, it can be seen that several of the bands appear to be "bottoming out" even though the apparent maximum absorptance is less than 10%. One possible reason for this effect is that the solute is not deposited as a uniform layer over the inside of the tube, but rather forms very narrow streaks of material at high concentration when the solvent evaporates, presumably due to the surface tension of the solvent. (For the solvent with the highest surface tension, the droplets could never be totally evaporated in the light-pipe and no solute was deposited). If only a very small fraction of the surface of the light-pipe has sample present, most of the radiation reflected down the tube would not interact with the sample. Confirmatory evidence for this hypothesis was obtained by using an uncoated glass tube, rather than a gold-coated light-pipe, for the deposition step. In this case very narrow streaks of sample could indeed be seen on the interior surface after spraying. We could find no way of improving the deposition characteristics of this method and we have reluctantly abandoned what initially appeared to be a very promising technique.

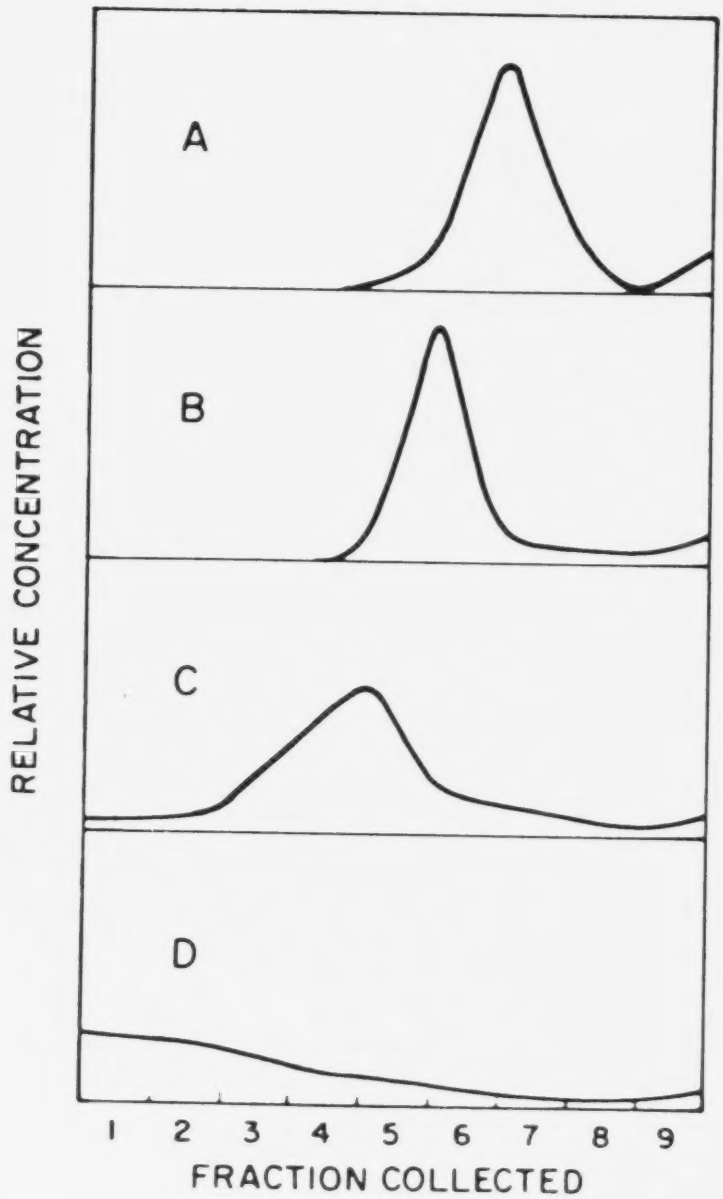

Figure 19A. Distribution of deposited solute down light-pipe with a nitrogen flow-rate of 2 cfm for the following voltages across the heating coil: A, 13v; B, 11v; C, 9v; D, 6v. (A,B,C and D corresponded to Variac settings of 12, 10, 8 and 6, respectively).

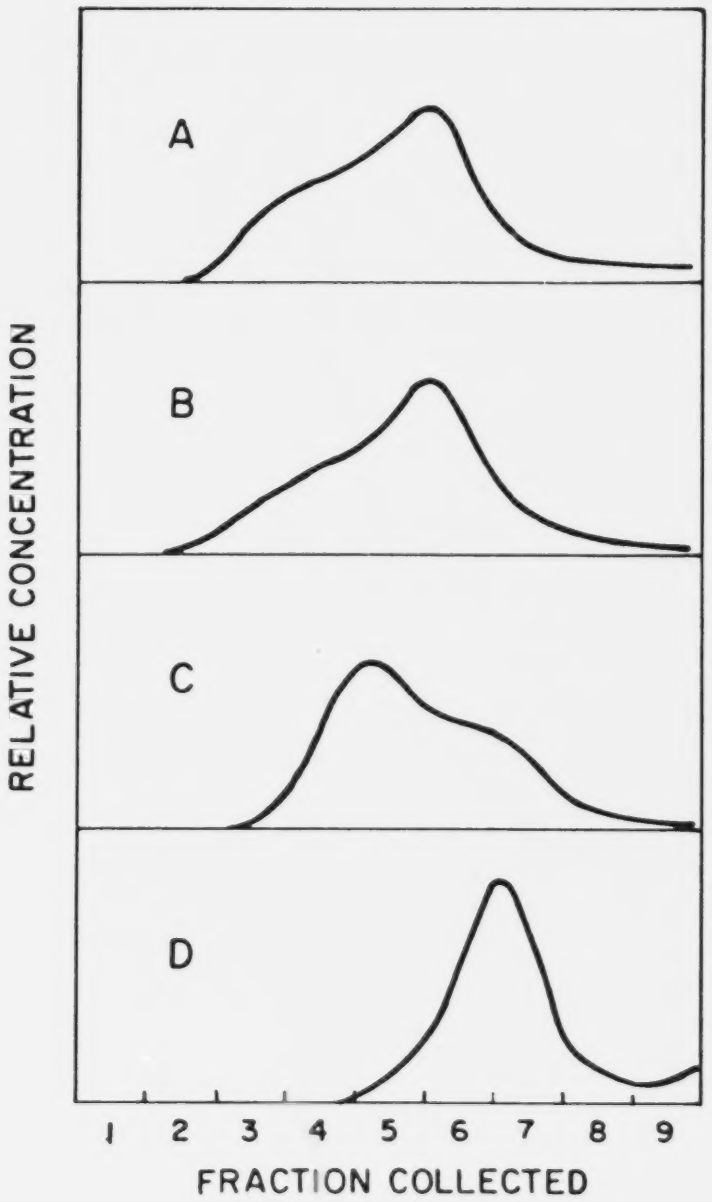

Figure 19B. Distribution of deposited solute down light-pipe with a voltage of 11v across the heating coil for the following nitrogen flow rates: A, 6 cfm; B, 5 cfm; C, 4 cfm; D, 3 cfm.

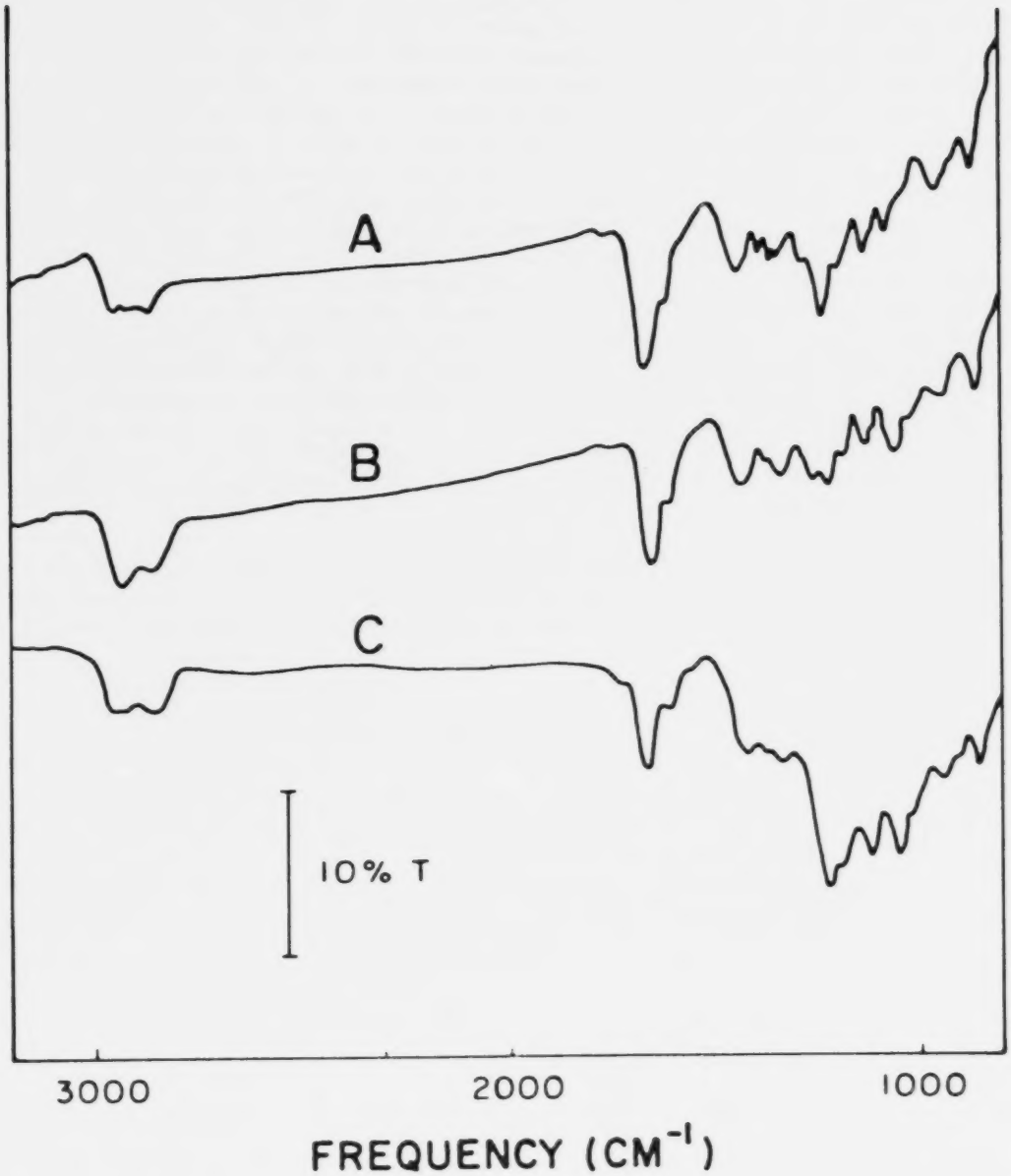

Figure 20. Spectra of 200 μg of methylene blue deposited on light-pipe, measured under the following conditions:
A. 3 mm light-pipe, 12% T screen in beam;
B. 3 mm light-pipe, central 90% of beam blocked;
C. 1.5 mm light-pipe, no further screening.

Some recent developments in this laboratory have finally led to a method which, at the point of writing this report, still appears to be a potentially powerful method for semi-continuous LC-FT-IR with solvent elimination. The method is based on diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy, a technique which was being developed in our laboratory for a purpose completely unrelated to this project. Our system is a hemiellipsoidal diffuse reflectometer, which functions because a beam of light emanating from one focus of an ellipsoid is reflected from the surface of the ellipsoid through the second focus. A beam of radiation is passed into the ellipsoid through a hole at the surface on the major axis and focused onto a diffuse reflector held at the nearest focus to the hole. The diffusely reflected radiation is collected by the ellipsoidal mirror and focused at the second focus of the ellipsoid. If the input beam is modulated by a rapid-scanning interferometer and a detector is held at the second focus of the ellipsoid, the diffuse reflectance spectrum of the sample held at the first focus could be measured. An optical schematic of our DRIFT apparatus is shown in Figure 21. In practice the magnification of the beam at the second focus is quite large and we use an off-axis segment of another paraboloid placed before this focus to reduce the size of the image to 2mm, the same size as our TGS and MCT detectors (24,25).

It has been found that finely powdered samples of alkali halides have a diffuse reflectance of nearly 100% (26). We found that if small quantities of strongly infrared absorbing compounds were mixed with powdered alkali halides,

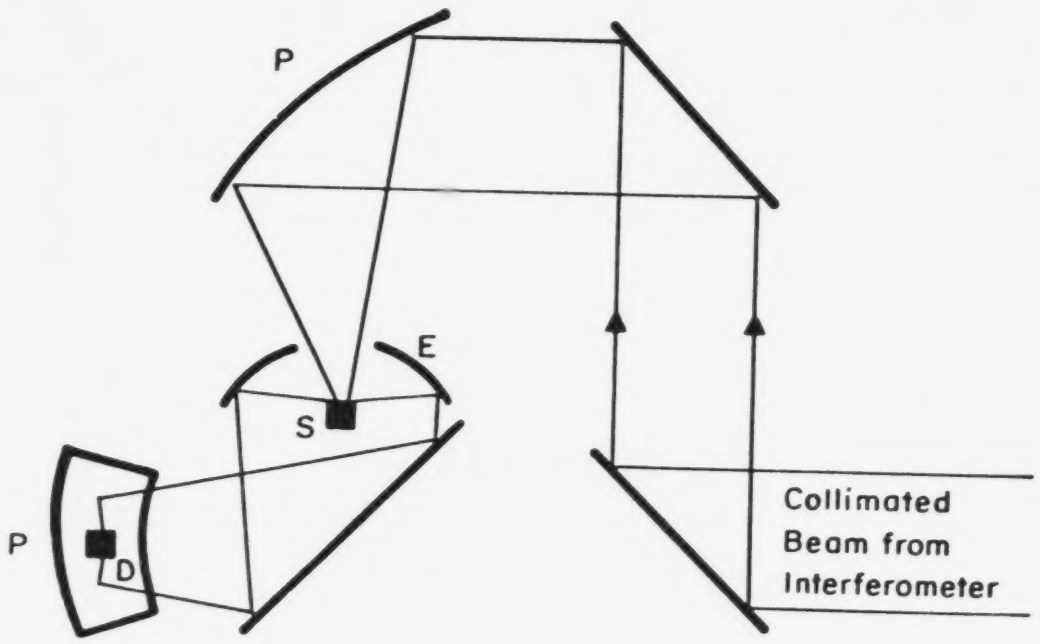

Figure 21. Optical diagram for hemiellipsoidal diffuse reflectometer.

and an MCT detector was used for the measurement, detection limits of a few nanograms were attained. We surmized that if a small quantity of powdered KCl were held on a fritted sample cup held at the first focus of the diffuse reflectance apparatus shown in Figure 21, it might be possible to evaporate the solvent from the HPLC effluent and deposit the solute on the surface of the KCl. This powder has a rather large total surface area, but can be held in a small (3mm diameter) cup; hence the sampling requirement for LC-FT-IR discussed above appears to be fulfilled. By holding several such cups in a carousel arrangement, a semi-continuous and completely automated LC-FT-IR system has been designed, and a "breadboard" system showing submicrogram detection limits has been constructed.

At least 30 sample cups are held around the circumference of the carousel at equal intervals. Each cup is about 3 mm deep and 5 mm in diameter, and contains about 100 mg of finely powdered KCl. The cup is lined with a fine mesh, so that when a low vacuum is applied to the bottom of the cup through a flexible hose, air is drawn through the KCl powder, causing any volatile solvent also present in the cup to be removed by evaporation. Holes were drilled through the wheel along the line joining the center of each cup to the center of the wheel, each hole being equidistant from the center. A small light bulb is held above the carousel over one of the holes and a photodiode is positioned immediately below the same hole. The wheel may be rotated using a belt driven by a motor, and its position at any time is sensed by counting the number of holes which pass the photodiode after its initial position. Three cup positions are used for sample deposition, final solvent elimination and spectral measurement, respectively, as shown in Figure 22 for a sixteen-cup carousel, and the whole device is controlled by a microcomputer.

The effluent from the liquid chromatograph is sprayed continuously into a heated concentrator tube, as shown in Figure 23. The concentrator tube is a thin glass tube wrapped with nichrome heating wire in such a fashion that the number of turns of the wire is greater around the top of the concentrator tube than near the bottom, so that the heat input to the spray is greater near the top of the tube where most of the solvent evaporates than near the bottom of the tube. The temperature of the interior of the tube is controlled by adjusting the voltage across the nichrome wire using a Variac so that about 90% of the solvent evaporates in the tube. If the chromatographic flow rate is 1 ml/min, the flow rate of the droplets emerging from the bottom of the tube is only 100 µl/min. The typical volume of solvent between the half-width points of an HPLC peak is about 30 seconds, so that if only this fraction of each peak is dropped onto the KCl sample cup, only 50 µl of solvent must be evaporated for complete elimination.

Initially a three-way solenoid valve was used to direct the effluent from the HPLC either to the concentrator tube or to waste, depending on whether or not a peak was eluting from the chromatograph. However with this arrangement the temperature of the concentrator tube would increase too much in the regions between eluting peaks, and a steady state condition was preferable. To achieve this end, the effluent was continuously sprayed into the concentrator tube, and a small capillary tube which is attached to an aspirator is placed at its tip so that all the liquid emerging is drawn down the capillary. A normally-open solenoid valve is held between the aspirator and the capillary, and when this

is in the closed position the emerging liquid is allowed to drop onto the KCl powder in the cup below. The solenoid is actuated by a trigger actuated from the HPLC detector after a preset delay to allow the peak to travel from the detector to the tip of the concentrator tube.

When the first peak eluting from the chromatograph is sensed, it is assigned a label by the microcomputer as are all peaks which elute subsequently. The status of each peak is listed in a table and continuously monitored. After the first peak has eluted from the concentrator tube, the solenoid valve is reopened to prevent any more solvent depositing on the KCl, and the wheel is rotated so that the cup containing this peak (cup A) reaches the solvent elimination position. At this point a stream of air is drawn through the cup so that all the solvent is evaporated. When the second peak reaches the tip of the concentrator tube, the solenoid is closed so that this peak is deposited onto the cup which is now at that position (cup B). At this time, we are allowing air to be drawn through cup A from the moment it reaches the solvent elimination position to the time

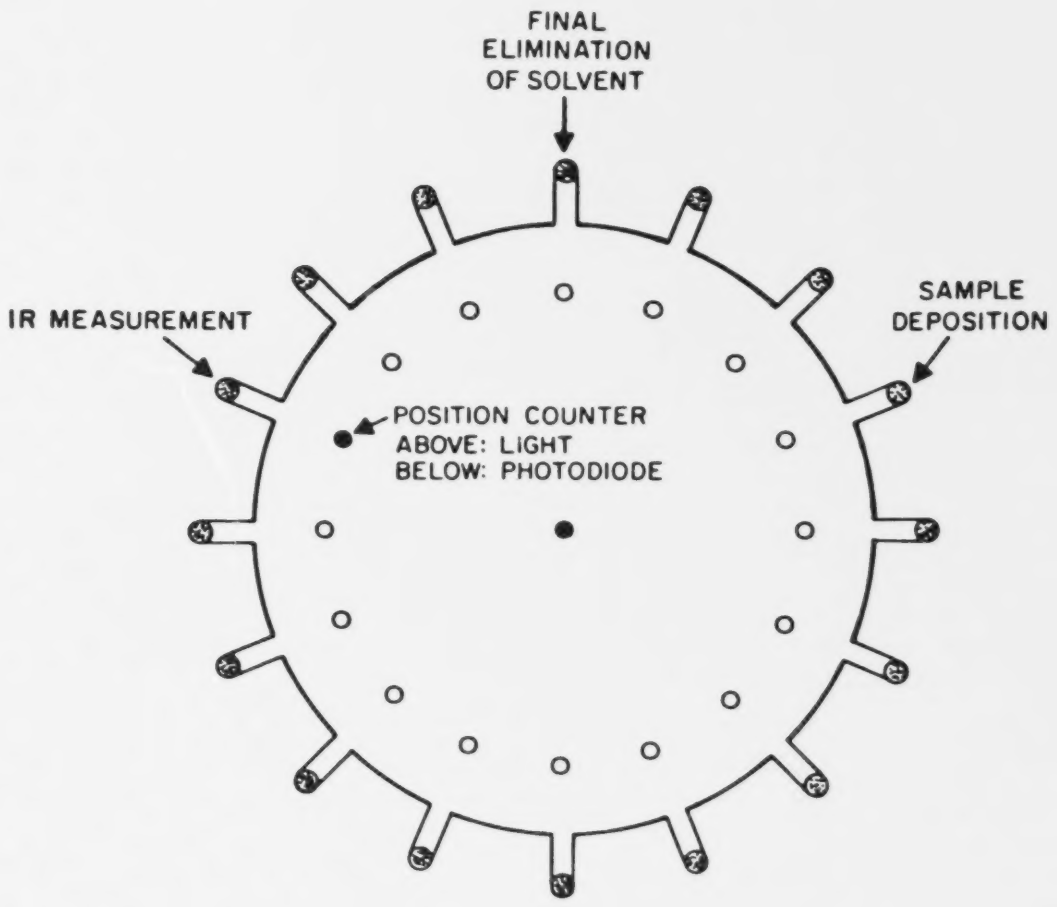

Figure 22. 16-cup carousel for LC-IR measurements by DRIFT spectrometry.

the next peak has been deposited into cup B. However we believe that it will be possible to reduce the time during which air is being passed through the cup in the solvent elimination stage in order to reduce solute loss by evaporation while still totally eliminating the solvent. Further work to optimize this stage will be performed. After the second peak has eluted, the solenoid valve is reopened to direct the flow of liquid emerging from the concentrator tube back to the aspirator and the carousel is again rotated. Cup A is then in the spectral measurement position, cup B is in the solvent elimination position, and the third peak is deposited in cup C.

The diffuse reflectance spectrum of the sample in cup A is measured from the time the carousel stops (if necessary after a short delay to allow all vibrations to die out) until the third peak is deposited in cup C. The micro-

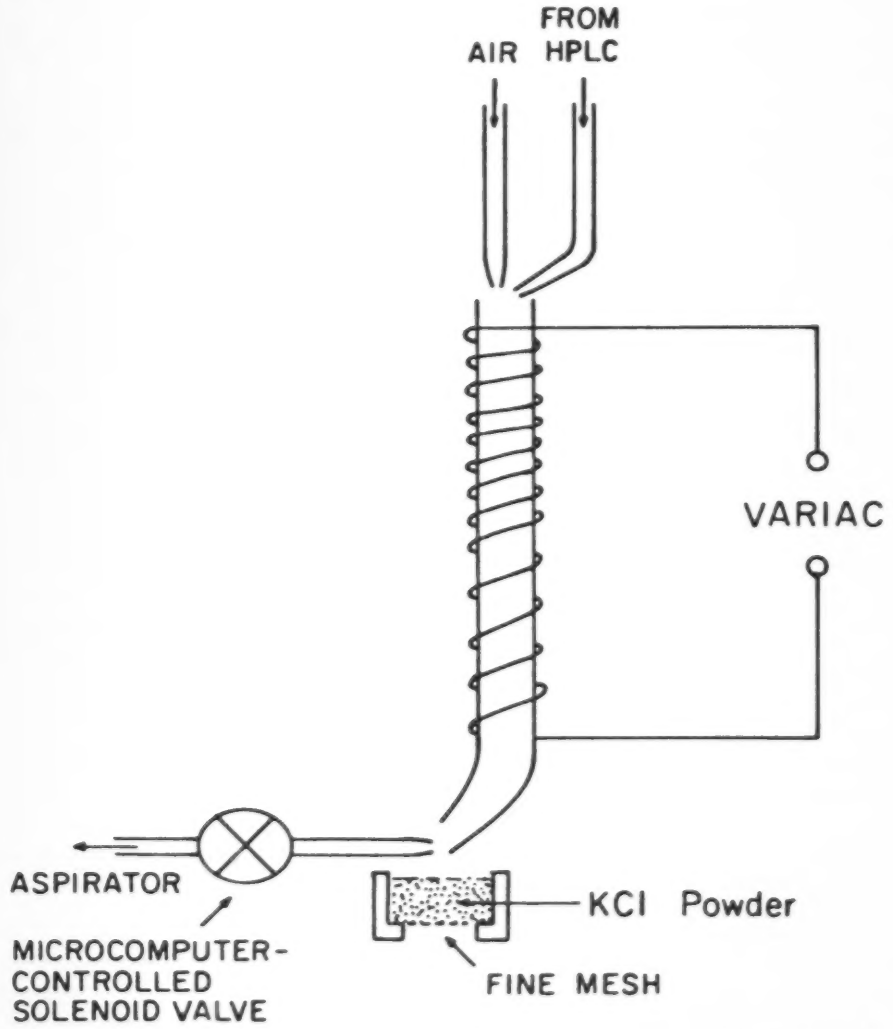

Figure 23. Concentration and solution deposition stage for LC-IR measurements by DRIFT spectrometry.

computer then waits for the scan currently in progress to finish and again actuates rotation of the carousel. This process is repeated each time a new peak is sensed at the HPLC detector, and is completely automatic. Two alternative methods are available for rotating the carousel to allow the cups containing the last two peaks to reach the spectral measurement position. The total number of peaks known to be present in the chromatogram can be preprogrammed into the microcomputer and the spectra of the samples in the last two cups can each be measured for a certain time, say two minutes. Alternatively a character can be entered into the keyboard of the microcomputer indicating that the last HPLC peak has eluted; in this case the microcomputer will control the carousel rotation and data acquisition in an exactly analogous way to the first method. The first method has the advantage of requiring absolutely no interaction but requires a chromatogram of that sample to have been run previously so that the operator knows the number of peaks. The second method does not require prior knowledge of the chromatogram; however the operator must be able to use some intuition to estimate when the last significant peak in the chromatogram has eluted.

The first"breadboard" system to be constructed certainly demonstrated the feasibility of achieving submicrogram detection limits. The sensitivity of this system was demonstrated using a 1 μl injection of Stahl's Test Dye Solution (Malinckrodt Chemical Co., St. Louis, MO), which is a solution of 0.1% each of three dyes, Butter Yellow, Indophenol Blue and Sudan Red G. The chromatograph used for this work was made by Tracor Instruments, Inc. (Dallas, TX), and was equipped with a 254 nm ultraviolet detector and a 25 cm long x 4 mm diameter column packed with 10 μm particle size Partisil, which is a silica adsorbent. The separation involved a normal-phase mechanism with a 99:1 hexane-propanol mixture as the mobile phase. Each component of the injected dye mixture was present at a total quantity of 1 μg, and the diffuse reflectance spectra of the three dyes, which are shown in Figures 24-26, show a S/N of better than 100. These spectra were measured using a completely unpurged spectrometer, and the effects of uncompensated atmospheric water vapor can be seen in these spectra between 1900 and 1700 cm^{-1}. The "derivative-like" shape of these water lines is caused by a slight difference in the alignment of the cups containing the samples and the cup containing the reference KCl. Misalignment of the beam in FT-IR spectrometry produces a wavelength shift and it became apparent that the tolerances to which our first carousel was made were not good enough for this work. A second carousel, made to far more exacting tolerances, is now being constructed. A cover to allow water vapor to be purged from the optical train is also being constructed. When this system is completed, we expect that detection limits of less than 10 ng of sample injected into the chromatograph will be attainable for nonvolatile solutes.

An interesting property of diffuse reflectance spectroscopy is beneficial for infrared microsampling in general, and for LC-IR in particular. The S/N is given by the ratio of $(1-R_\infty)$ for any absorption band to the noise at the same wavenumber, where R_∞ is the reflectance of the sample at "infinite depth". (We have shown (24) that the reflectance changes very little on increasing the depth beyond 3 mm). According to the Kubelka-Munk equation (27) linking R_∞ to the concentration, c, of sample in a nonabsorbing matrix such as KCl:

$$\left(\frac{1-R_\infty}{2R_\infty}\right)^2 \quad \alpha \quad kc$$

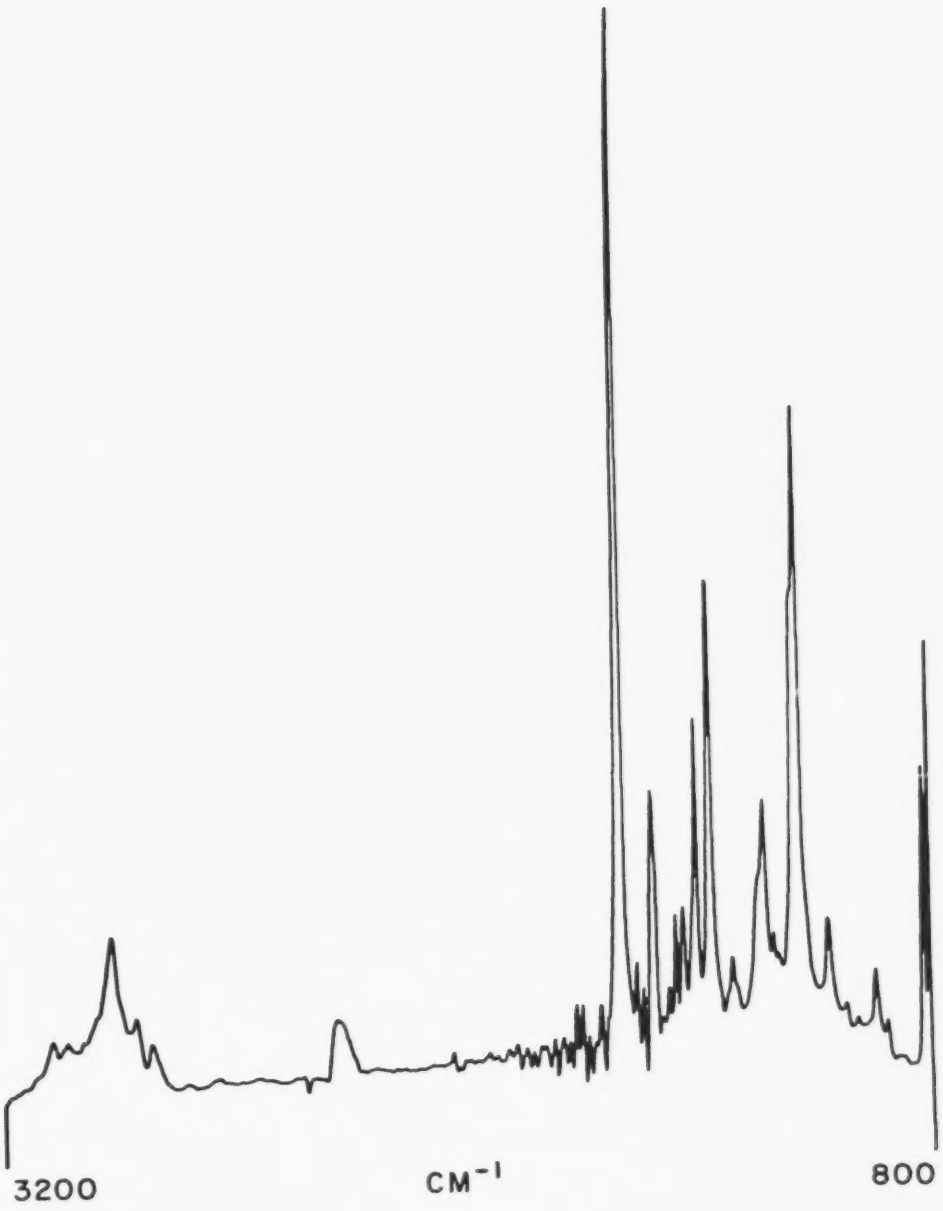

Figure 24. On-line LC-IR spectrum of 1 μg of Butter Yellow dye, measured after automatic solvent elimination.

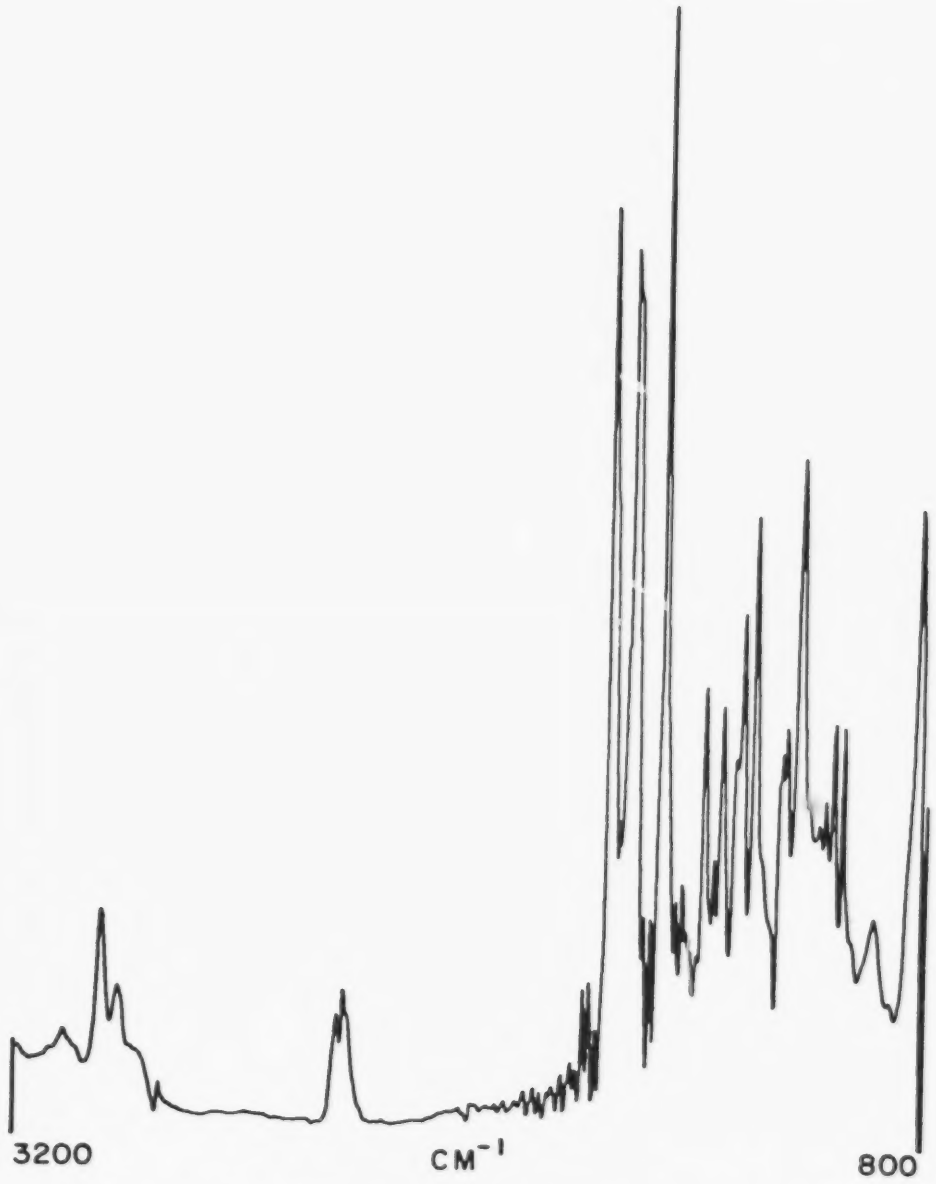

Figure 25. On-line LC-IR spectrum of 1 μg of Indophenol Blue dye, measured after automatic solvent elimination.

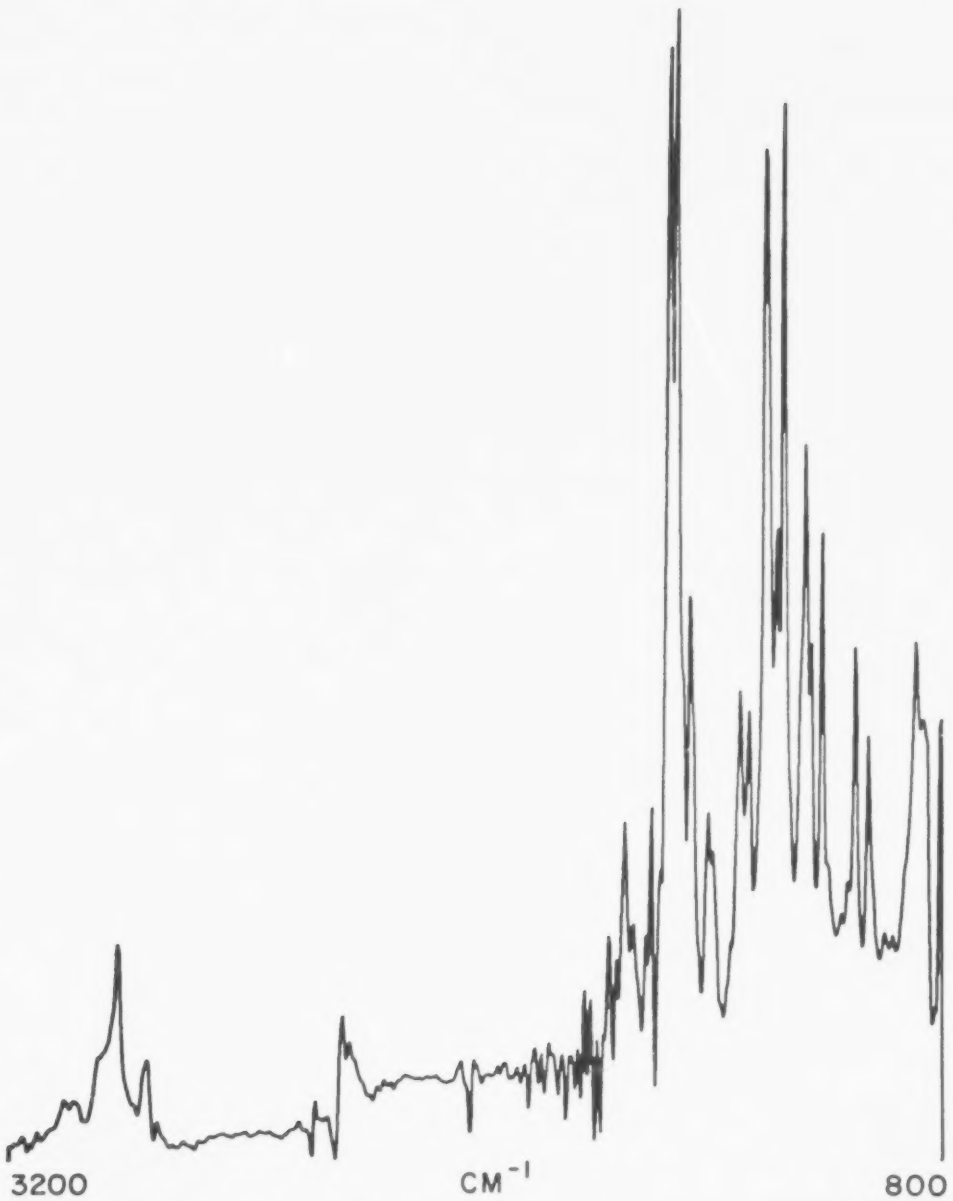

Figure 26. On-line LC-IR spectrum of 1 µg of Sudan Red G dye, measured after automatic solvent elimination.

where k is the absorption index, which is directly proportional to the absorptivity. When c is small, R_∞ becomes approximately unity, so that the denominator of this expression becomes constant, and the signal, $(1-R_\infty)$, becomes proportional to the square root of the concentration. In almost every other spectroscopic technique, the S/N becomes linearly proportional to the sample concentration near the detection limit, and this property should give DRIFT a real advantage in infrared ultramicrosampling compared with other more conventional sampling techniques.

Although this LC-FT-IR method is obviously at a very early stage in its development it shows real promise, and even at this stage shows detection limits two orders of magnitude below methods in which the solvent is not eliminated and the HPLC effluent is passed directly through a flow-through cell. This sensitivity improvement is measured in regions where the solvent is transparent, and substantially greater improvements are found in the regions of strong solvent absorption. The method is of course, applicable even for gradient elution HPLC, thereby obviating the need for the extremely difficult solvent compensation routines needed when flow-through cells are used in this case. Another advantage of the technique is that each peak is captured, and if the S/N is insufficient to allow unequivocal identification, the cup may be readily returned to the spectral measurement position and the spectrum may be measured using more extensive signal-averaging than is possible in this automated measurement. As yet we have been unable to totally eliminate aqueous solvents, largely because of the high surface tension and latent heat of vaporization of water. We believe that there are several other ways of approaching this problem which have not yet been tested, and we are not rejecting the idea that aqueous mobile phases cannot be separated from the solute. However this type of chromatography is equally difficult to investigate by conventional LC-FT-IR techniques because of the extremely high absorptivity of water all through the spectrum. Overall the LC-FT-IR method which we have developed appears to be a promising, and perhaps even a very powerful, method for the on-line measurement of the infrared spectra of peaks eluting from high performance liquid chromatographs.

SECTION 8

THIN-LAYER CHROMATOGRAPHY AND FT-IR SPECTROSCOPY

TRANSMITTANCE MEASUREMENTS

We have previously developed a method by which the infrared spectra of species separated by thin-layer chromatography (TLC) can be measured without removing the sample from the adsorbent (23,29). In this technique the adsorbent layer (silica gel or alumina) is deposited on an insoluble, chemically inert, infrared transmitting plate (AgCl) so that after the chromatogram has been developed, the absorption spectrum of each separated sample "spot" can be measured by direct transmission. The plate may be treated with a small quantity of Fluorolube (whose refractive index closely matches that of the silica or alumina) prior to the infrared measurement to reduce the effect of scattering at high wavenumber (29). The sensitivity of this technique, which we abbreviate as TLC-IR, is determined in part by the spectrometer and in part by the chromatography.

The sample absorbance of TLC-IR spectra depends on the number of analyte molecules per unit area of surface. Until the spot size becomes smaller than the area of the infrared beam at its focus (typically 3mm), the smaller the diameter of the spot can be made, the higher is the sample concentration and the higher is the infrared absorbance. One of the limitations of conventional TLC is that spots usually spread to a greater diameter than 3 mm during development, thereby reducing the potential sensitivity of TLC-IR measurements. The use of programmed multiple development (PMD) in TLC decreases the spot size and increases chromatographic resolution (30-32).

PMD is a form of TLC in which the chromatography is performed using several developments, each one allowing the solvent front to advance further than the preceding one. A controlled solvent removal step, either using radiant heat or by sweeping the plate with an inert gas, follows each solvent advance returning the solvent to the spot origin. Each cycle consists of a solvent advance (development) and a solvent removal (drying) step, with the development time during the n th cycle, t_n, depending on n and the advance unit time, t_a. With our equipment (Regis Chemical Company, Morton Grove, IL), any of the following modes may be selected:

Mode 1: $$t_n = n\, t_a$$

Mode 2: $$t_n = \sum_{i=1}^{n} i t_a$$

Mode 3: $t_n = n^2 t_a$

where t_a ranges from 10 to 100 seconds. Mode 1 yields the most cycles for a given overall program time and best resolves spots of low R_f value. Mode 3 most closely resembles conventional TLC, yields the fewest cycles for a given overall program time, and best resolves spots of high R_f value. Mode 2 is intermediate between Modes 1 and 3.

PMD has three major advantages over conventional TLC for TLC-IR measurements. The first is the increased chromatographic resolution as mentioned above. In addition, the original solution may be applied to the plate in greater volume and over a larger area than for conventional TLC before the spot size starts to increase. Finally the adsorbent layer may be loaded with a much greater quantity of sample than conventional TLC before the chromatographic resolution becomes degraded. Since the thickness of the layer for TLC-IR experiments is ideally between 50 and 100 μm (to avoid excessively strong absorption bands due to adsorbent) compared to the usual range of 100 to 250 μm for many commercially available TLC plates, the probability of exceeding the adsorbent capacity for conventional TLC is increased, and the use of PMD should be beneficial when TLC-IR measurements are desired.

TLC plates for this work were prepared by depositing a layer of alumina (mean grain size, 10 to 40 μm) on a silver chloride plate, as described previously (28,29). After development by conventional TLC or PMD, the plate was treated with Fluorolube and held at the 3 mm sample focus in the transmitted beam of the dual-beam FT-IR system shown in Figure 6 with the reflected beam blocked. This measurement is equivalent to holding the sample at the focus of a 4x beam condenser in the sample compartment of an unmodified Digilab FTS-14 spectrometer, but allows larger plates to be studied without vigenetting the beam.

Two types of compound, dyes and chlorinated pesticides, were investigated. The dyes were methylene blue and Stahl's solution (an equimolar solution of butter yellow, Sudan red G, and indophenol blue). Visualization of the spots of separated pesticides was achieved by exposure of the dried plate to iodine vapor. All spectra were measured at 8 cm^{-1} resolution in double-precision, using either the TGS or MCT detector.

To compare the sensitivity of TLC-IR experiments performed by conventional TLC and PMD, two identical chromatoplates were prepared, spotted with 500 ng of the pesticide, Aldrin, and developed with n-heptane. For the PMD separation, Mode 1 was used with n = 15 and t_a = 10s, giving a total time of 30 minutes, compared with 15 minutes for conventional TLC. The area of the spot on the PMD chromatogram was approximately five times less than that for the conventional TLC plate. The measured spectra are shown in Figure 27, and the improvement in sensitivity for the PMD run is apparent.

The advantage of improving the chromatography by PMD and increasing the spectrometric sensitivity by substituting the TGS detector by an MCT detector is illustrated in Figure 28, for butter yellow dye separated from Stahl's solution. The increase in concentration gained through the use of PMD is

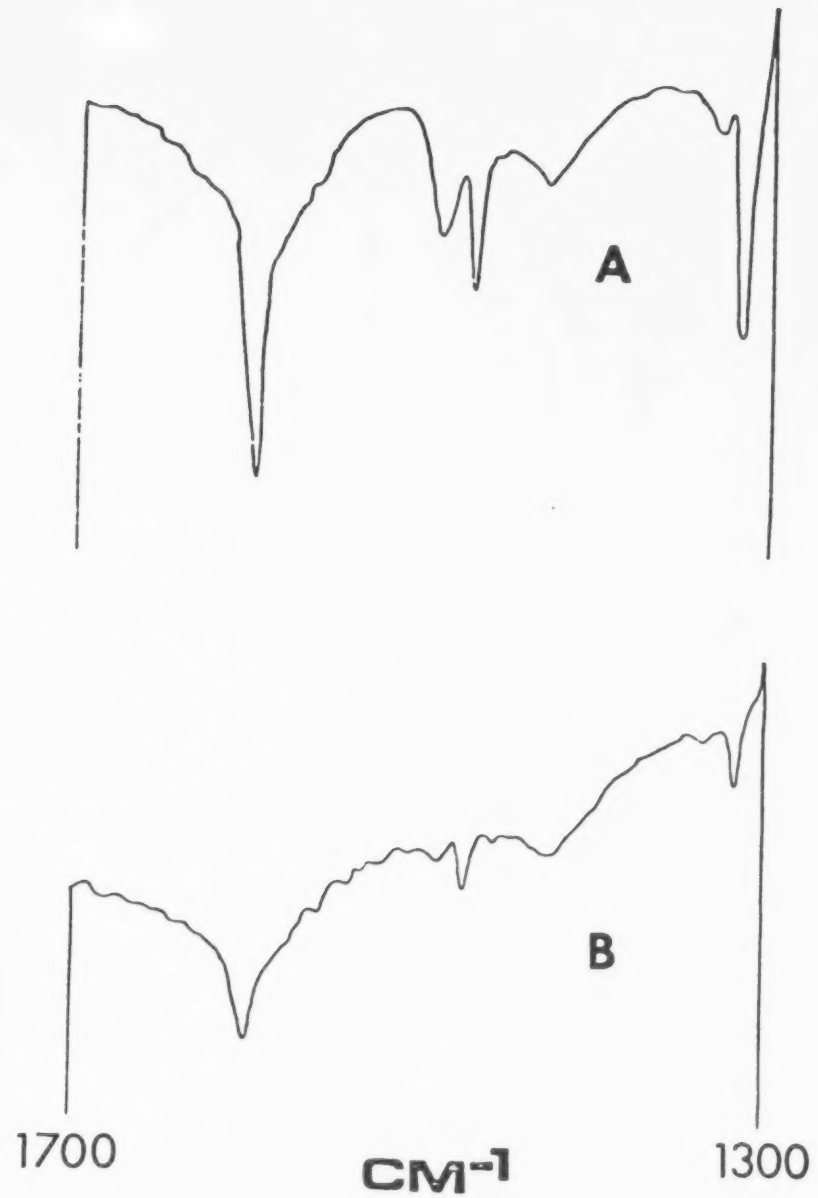

Figure 27. TLC-IR spectra of 500 ng of Aldrin measured with an MCT detector using 10 scans:
A. by PMD, Mode 1, n=15, t_a=10 s, total development time = 30 min;
B. by conventional TLC, development time = 15 min.

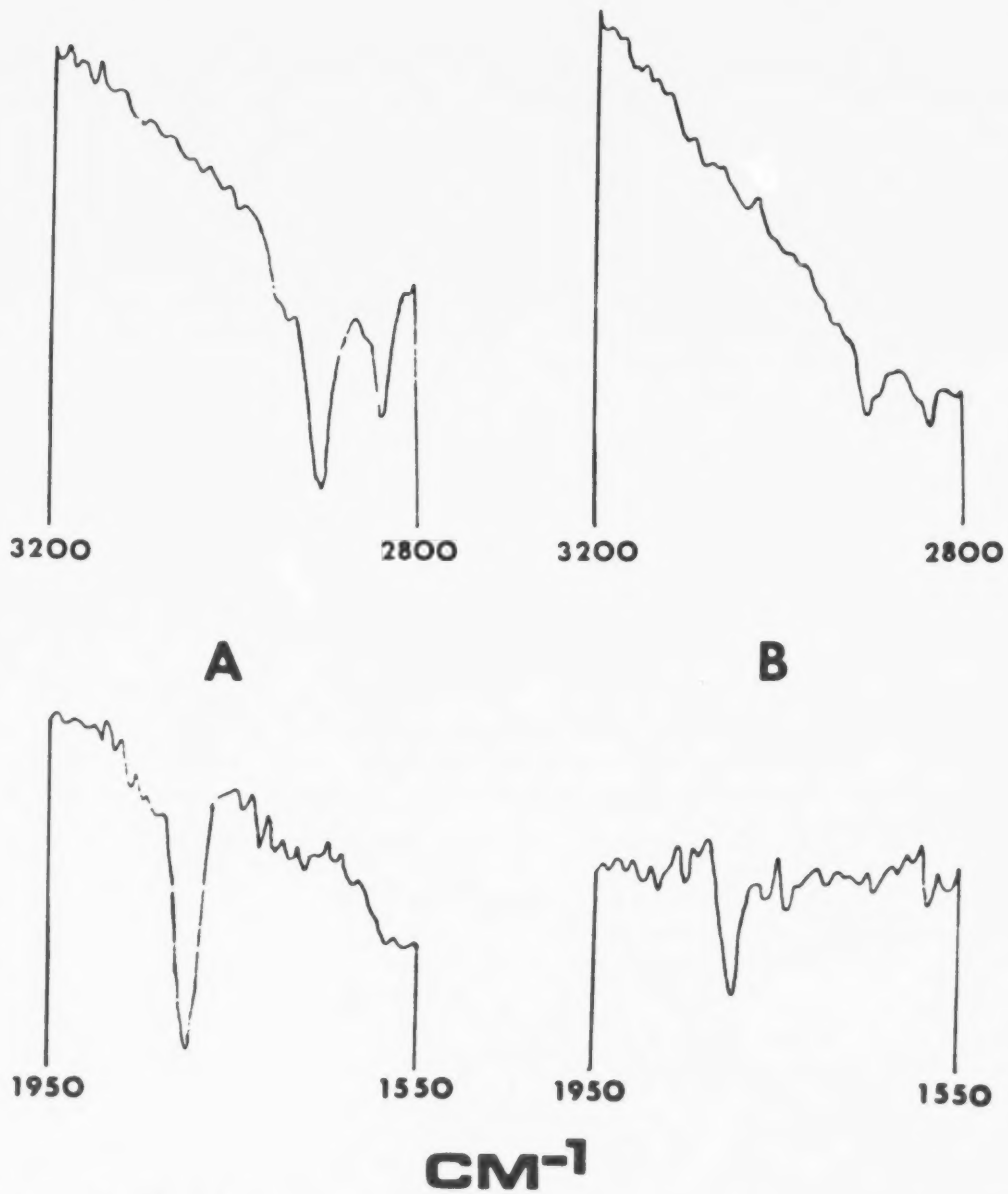

Figure 28. Portions of TLC-IR spectra of Butter Yellow after separation from the other components of Stahl's solution, using benzene as eluent:

A. 100 ng of sample separated using PMD, Mode 3, n=6, t_a=10 s, total development time = 45 min, measured with an MCT detector using 10 scans;

B. 500 ng of sample separated by conventional TLC, development time = 15 min, measured with a TGS detector using 400 scans.

shown by the increased absorbance of the bands, while the advantage of using the MCT detector is reflected in the reduced measurement time required to achieve a given noise level.

It is noteworthy that when separation is effected by PMD and an MCT detector is used for the infrared measurement the total (active) scan time required to attain submicrogram sensitivity is less than 5 seconds, even for such weakly absorbing samples as the chlorinated pesticides. These results suggest that it may be feasible to construct a device to measure TLC-IR spectra automatically across an entire chromatoplate developed by PMD. Spectra could be measured after moving the plate by intervals as small as 1 mm in a total measurement time less than the time taken to develop the plate. The only unfavorable factor for such a device would be the difference between the shape of the infrared beam at its focus (circular) and the shape of spots separated by PMD, which are elongated ellipses, typically approximately 1 by 6 mm. There are two ways to improve this situation. It might be possible to use a toroidal mirror rotated 90° through its optical axis to deform the beam to the desired shape as suggested by Low (33). It has also been shown that it is possible to modify the PMD experiment to obtain circular spots (31).

If PMD is used for TLC-IR measurements, detection limits as low as 10 ng can be achieved with extended signal-averaging, as shown in Figure 29. It may be noted that since the diameter of the infrared beam is larger than the minor diameter of the elliptical spot on the chromatoplate, a little over half the beam misses the sample completely. Thus if better matching could be achieved by either of the techniques discussed above, the detection limits should be further reduced by about a factor of two.

DIFFUSE REFLECTANCE MEASUREMENTS

The only major disadvantage of the TLC-IR measurements described above is that chromatoplates on AgCl substrates are not commercially available. Commercial TLC plates are made on either glass, plastic or metal foil backings. None of these materials is sufficiently infrared transmitting to be usable for TLC-IR measurements by direct transmission. We therefore decided to test the feasibility of diffuse reflectance spectroscopy for TLC-IR measurements. One advantage of DRIFT for this application is that it is not necessary to treat the plate with Nujol or Fluorolube after development to eliminate the effects of scattering at high wavenumbers, since the technique only works for scattering samples. On our particular system for DRIFT measurements (Figure 21), we can only measure samples of 4 mm diameter, so that TLC-IR spectra cannot be obtained without removing the sample from the plate in some fashion. We are able to punch out small samples from TLC plates on plastic or metal foil backings and place the spots at the sample focus to investigate the feasibility of using DRIFT for TLC-IR measurements.

For silica TLC plates on polyethylene terephthalate backings (Eastman Chemical Company, Rochester, NY) we found that the penetration depth was greater than the thickness of the silica layer (100 μm) so that the polymer bands were very evident in the measured spectra. We therefore investigated the feasibility of using aluminum foil backed silica TLC plates (Brinkmann Instruments, Hauppage, NY). Several problems were encountered in this work.

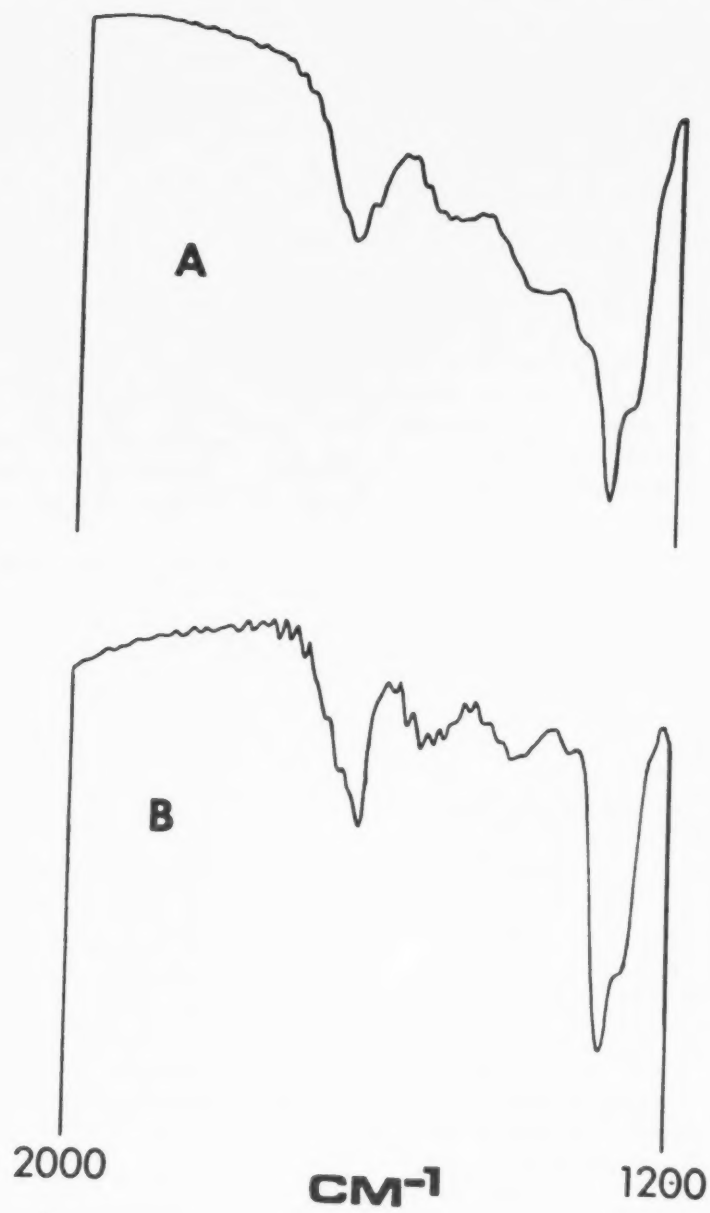

Figure 29. TLC-IR spectra of methylene blue developed by PMD using methanol as eluent, Mode 1, n=10, t_a=10 s, measured using an MCT detector:
A. 100 ng of sample, 10 scans;
B. 10 ng of sample, 1000 scans.

Firstly it was found that the diffuse reflectance from silica, and other materials with bands of unusually high absorptivity, is strongly dependent on the particle size (25). When the particle size is between about 100 μm and 10 μm, the spectrum appears to be a combination of the specular reflectance spectrum and the diffuse reflectance spectrum, with the proportion of the diffuse reflectance spectrum increasing as the particle size is decreased. For particles of approximately 40 μm diameter, the bands which would normally show as pure absorption bands in diffuse reflectance spectra appear to insert near the band center because of the specular reflectance component. The specular component of the beam does not penetrate the sample at all so that even though there appears to be enough energy across the entire spectrum that absorption bands of sorbed species should be measurable at all wavenumbers, these bands are in fact masked near the maxima of the adsorbent bands. Only by using an adsorbent with an average particle size considerably less than 10 μm could one expect to make the contribution of the specular reflectance component negligibly small.

The second problem concerned the feasibility of adequately compensating the bands due to water associated with the silica gel. The intensity of these bands would change before and after chromatography, so that it was necessary to use a region of the chromatoplate close to that of the spot being studied to obtain a good matching of the intensity of the water bands and hence to obtain a good baseline in the compensated spectrum. We were never able to obtain a perfect baseline in any TLC-IR measurement by DRIFT, although several spectra with less than 5% deviations around the 100% reflectance line were obtained.

Finally if an alcohol, such as methanol, was used to develop the chromatogram, reaction between the silanol group on the silica gel and the methanol was shown to occur:

$$\equiv SiOH + CH_3OH \rightarrow \equiv SiO\,CH_3 + H_2O$$

The extent of this reaction was dependent on the R_f value of the spot, so that once again great care had to be taken in the compensation of the background absorption.

In spite of all these problems, we were able to measure spectra of materials sorbed on silica-gel TLC plates after elution with a variety of solvents with submicrogram detection limits, see Figure 30. The difficulties involved with background compensation make it appear that the development of an on-line scanner for TLC plates based on diffuse reflectance spectrometry would be of marginal use to analytical chemists involved in trace organic analysis.

Figure 30. TLC-IR spectra of 1.2 μg of methylene blue measured by DRIFT spectrometry. About one half of the observable bands on this spectrum are caused by the adsorbate, and the rest are caused by the silica gel adsorbent.

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